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ACUTE PORPHYRIA

EXPERIMENTAL TREATMENT WITH A.C.T.H.

BY

A. GOLDBERG, M.B., F.R.F.P.S., M.R.C.P.
Medical Registrar, Western Infirmary, Glasgow

A. CAMERON MACDONALD, M.B., F.R.F.P.S. M.R.C.P.
Senior Medical Registrar, Western Infirmary, Glasgow

AND

C. RIMINGTON, M.A., Ph.D., D.Sc.
Professor of Chemical Pathology, University of London

In the past two decades the clinical study of the porphyrias has received growing attention. The aetiology of these diseases, however, remains obscure and their treatment symptomatic. It was perhaps inevitable that a new therapeutic agent, such as A.C.T.H., would be tried in the treatment of this puzzling group of diseases, particularly as it has been suggested that there may be some connexion with hypofunction of the adrenals. Myerson (1951) and Oltman and Friedman (1951) did not find A.C.T.H. of value in this condition. This paper reviews the clinical features of two cases of acute idiopathic porphyria, in one of which a course of A.C.T.H. was given.

Case 1

A man aged 28 was admitted to the Western Infirmary, Glasgow, on February 21, 1946, suffering from severe abdominal pain ; he gave a history of four previous attacks of abdominal and limb pain. His first bout was in September, 1944, when he was serving in the Royal Air Force, and his condition was diagnosed in an R.A.F. hospital as idiopathic porphyria. He had been a coal-miner before joining the R.A.F., and had had no previous ill-health. There was no family history suggestive of porphyria.

Since February 21, 1946, six further attacks were observed in the Western Infirmary, and he was admitted on numerous occasions to Ayr County Hospital. Dr. R. Hill is publishing observations made on this case while under his care in the latter hospital. Some episodes of porphyria were characterized by abdominal pain only, but several showed both

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colicky pain and polyneuritis. They varied in duration from a few days to a month.

A typical bout observed in the Western Infirmary and beginning on September 22, 1946, had the following features. While in apparently good health, the patient noticed that his urine became darker in colour. Within a few hours generalized abdominal pain developed; the pain was continuous and was punctuated by frequent bouts of colic of great severity. At this stage the abdomen was as rigid as in a case of perforated peptic ulcer. He was constipated and his urine became scanty and reddish black in colour. A persistent tachycardia up to 150 a minute was present, and the blood pressure rose to 170/130 (his normal blood pressure was 120/90). Within one week the abdominal pain subsided and was succeeded by pain in the thighs, with marked spasm of the abductors and hamstrings. The pain spread to involve the muscles of all four limbs, which showed hypotonicity, pronounced wasting, and diminished tendon reflexes. No loss of vibration or tactile sense was noted, but the muscles were very tender. This polyneuritic phase lasted three weeks. The pain gradually lessened during the final week, and this was accompanied by reduction in the intensity of the urinary coloration. Convalescence was rapid when the pain subsided.

During the acute stages of a few attacks mental confusion and hallucinations were noted. Staphylococcal skin infections were common, and in one episode bronchopneumonia developed (September 10, 1948). The patient stated that during his attacks he tended to become "blue in the face." Body weight fell by about 30 lb. (13.6 kg.) during each attack, but weekly records failed to demonstrate any weight loss before the onset of pain.

Investigations.—Repeated clinical and laboratory investigations were carried out during the attacks, and the following abnormalities were found: (1) The patient continuously excreted uroporphyrins, almost entirely the Series III isomer, with a marked rise in excretion during acute attacks. The isomer type was established by partial decarboxylation and separation of the resulting mixture of coproporphyrins. (2) The plasma chlorides fell to levels around 0.52 g.% (as NaCl) during attacks. (3) Liver-function tests showed some impairment of hippuric acid synthesis.

Treatment of the acute attacks by intravenous calcium gluconate, by saline and glucose–saline, or by the injection of whole adrenal extracts ("eucortone"), of D.C.A., and of B-group vitamins produced no significant improvement. The symptomatic treatment of pain required large doses of morphine-type drugs over long periods.

At the time of writing the patient's condition is satisfactory, though his urinary porphyrin excretion is still above normal and includes uroporphyrin. Recently the episodes have consisted of mild attacks of abdominal discomfort of

a few hours' duration, occurring every three to four months. The attacks are adequately controlled by minor analgesics, and he is able to work as a storeman.

Case 2

A single woman aged 21, a whisky-bond worker, was admitted to the Western Infirmary, Glasgow, on January 19, 1951, because of lower abdominal pain, nausea, vomiting, and constipation of two days' duration. The pain was constantly present, but became more severe and colicky at times. She had been normally active and healthy until two months before her admission to hospital, but since then her mother thought she looked "thinner in the face" and was not so active as previously. So far as could be ascertained she had taken no drugs in the past few months. There was nothing of note in the family history.

On admission the patient was anxious, was crying, and was in great pain. A noteworthy feature was that her lips, ear-lobes, and finger extremities were cyanosed and remained cyanosed for about three to four days. Her temperature was 100.2° F. (37.9° C.), pulse rate 140, blood pressure 150/115, and white blood count 8,200 per c.mm. General physical examination revealed no abnormal signs except some slight generalized abdominal tenderness. There was no rigidity of the anterior abdominal wall.

On the day after admission her abdominal pain remained very severe and she was still vomiting. It was noted that her urine was rose-brown in colour, and gave the spectrum characteristic of porphyrin. The reaction of the urine to Ehrlich's aldehyde reagent was strongly positive. The pain continued for two days, then diminished greatly in severity as she began to menstruate on January 22, 1951. For the next month she had little abdominal pain, but complained of shooting pains in the medial aspect of both thighs. She was up and about the ward, her blood pressure had returned to normal (115/80), and her urine was clear.

On February 19, about one month after her admission and just before her expected period, she started a prolonged and severe attack with almost constant abdominal pain, and with tachycardia, amenorrhoea, and the passage of red-brown urine. In this phase, lasting about six weeks, she lost 28 lb. (12.7 kg.) in weight. There was marked and progressive mental deterioration. She often screamed and shouted in the ward, had visual hallucinations, and threatened suicide. In order to provide relief she was given, on different occasions, opiates, chloral hydrate, paraldehyde, nicotinamide, diphenhydramine hydrochloride ("benadryl") methylene blue, kaolin, and intravenous calcium gluconate. With the exception of the opiates, chloral hydrate, and paraldehyde, these drugs did not seem to benefit her.

By March 27 her general condition had become very poor. She was weak, hypotensive (B.P. 70/54), somewhat dehy-

drated, and still in agony with abdominal pain. Up to this time she had required large doses of sedatives, and this aggravated the dehydration. She was given 1½ pints (850 ml.) of normal saline by a subcutaneous drip (using hyaluronidase, 9 mg.) and began a course of A.C.T.H. A daily dosage of 50 mg. was given for one week, in six-hourly injections of 12.5 mg. intramuscularly. Her general condition improved within one or two days, and her blood pressure rose to 120/100. She required less sedatives, and thus was able to drink more, and the urinary output improved. She became euphoric and excited at times and began to sing to the other patients. Her physical condition improved slowly, and, although she still passed reddish urine and was still depressed and unstable, she was allowed home on April 19. Three months later she was free from pain, had put on weight, and had almost returned to her normal mental state.

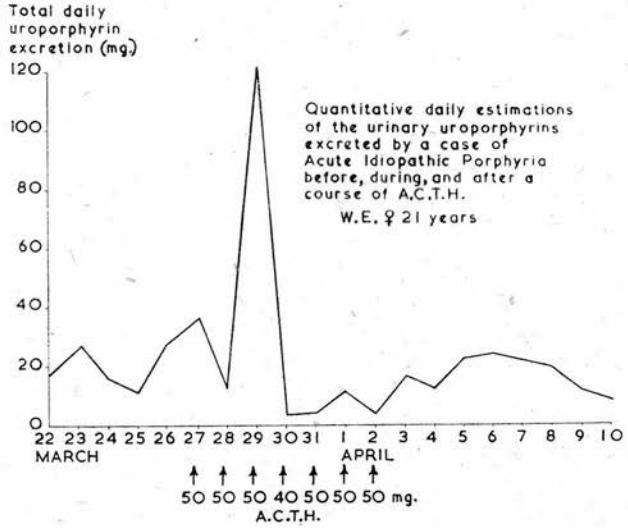
Investigations

Blood examination resulted as follows : Hb. 11.4 g.% ; red cells, 4,280,000 per c.mm. ; P.C.V., 43% ; M.C.V., 100 μ^3 ; M.C.H.C., 27% ; white cells, 7,400 per c.mm. (neutrophil polymorphs 80%, lymphocytes 17%, eosinophils 2%, basophils 1%) ; reticulocyte count less than 1% ; icteric index, 3 units ; E.S.R. (Westergren), one hour 4 mm., two hours 7 mm. Film showed no abnormality. The blood picture showed no change on repeated examinations during her stay in hospital. The Coombs test and red-cell-fragility test were normal. Repeated estimations of serum sodium and chlorides before, during, and after A.C.T.H. administration were normal. Alkaline phosphatase was 7 King–Armstrong units ; cephalin flocculation and colloidal gold tests were negative ; total plasma protein 6.85 g.% (albumin 4.57 g.%, globulin 2 g.%, fibrinogen 0.28 g.%) ; non-protein nitrogen, 36 mg. per 100 ml. ; blood urea, 34 mg. per 100 ml. ; van den Bergh reaction, negative ; blood cholesterol, 165 mg. per 100 ml. The electrocardiogram and the *x*-ray film of the chest were normal.

The adrenaline–eosinophil test (Recant *et al.*, 1950) was carried out on two occasions. On March 19 the eosinophil count changed merely from 248 to 246 per c.mm. four hours after the subcutaneous administration of 5 minims (0.3 ml.) of 1 : 1,000 adrenaline hydrochloride. This was repeated three days later and showed a drop from 181 to 137 per c.mm. four hours after the subcutaneous injection of 7 minims (0.42 ml.) of 1 : 1,000 adrenaline. After A.C.T.H. was given on March 27 her eosinophil count fell from 147 per c.mm. to zero in four hours.

The urine contained uroporphyrin, mainly of Series III. An amyl alcoholic extract of the urine was very fluorescent under ultra-violet light. Quantitative estimations of the total daily urinary uroporphyrin excretion before, during, and after A.C.T.H. therapy were carried out after prelim-

inary heating of the urine at *p*H 4.8 as described by Grieg *et al.* (1950). The results were as follows (see Graph):



Comment

These two cases conform to the usual clinical picture of acute idiopathic porphyria. In Case 1 there were numerous episodes over a period of seven years, and in the second case two attacks occurred in three months. Thus the condition is often a subacute or chronic one, though it may appear acutely in its presenting phase.

The age of onset—22 years in Case 1 and 21 years in Case 2—is typical of this disorder, which usually begins in the third decade. In both cases the presenting features were acute colicky abdominal pain associated with tachycardia and hypertension. Definite board-like rigidity occurred in Case 1 but not in Case 2. Grossfeld (1951) and Chandler *et al.* (1939) reported cases in which the abdominal signs were such that laparotomy was performed.

Pain in the limbs occurred in both patients, but manifest neurological signs were present only in Case 1. In this case mild mental confusion and hallucinations occurred during acute attacks. In Case 2, on the other hand, there was a pronounced toxic psychosis ; the patient's personality completely changed and only returned nearly to normal several months after the acute attack had settled.

During the acute phases weight loss was noted in both patients, but records taken in Case 1 failed to demonstrate the pre-episodic fall stressed by Discombe and D'Silva (1945).

Cyanosis of the extremities, and especially of the lips, was striking in Case 2, while Case 1 was described as becoming "blue in the face" during attacks. Similar cyanosis has been described by Turner (1938). Venous blood from Case 2, examined during this cyanosed phase, did not contain methaemoglobin, and a short course of methylene blue, given empirically, had no apparent clinical effect.

The dramatic fluorescence, under ultra-violet light, of urine containing porphyrins encouraged us to examine the skin of Case 2 under that light. Interest was aroused when tiny pin-points of intense rose-pink fluorescence were found on the naso-labial folds and on the anterior and posterior surfaces of the patient's chest. A few days later, however, it was found that the application of the proprietary talcum powder used by the patient produced similar fluorescence in a normal control. Abrahams *et al.* (1947) also failed to find skin fluorescence in their case of acute porphyria.

It is well known that a clinical state of hypotension with low serum sodium and chlorides may supervene in acute porphyria, although in some cases these changes are not observed. Abrahams *et al.* (1947) and Linder (1947) have suggested that this is due to adrenal insufficiency and have used eucortone in this phase, but without much success. On the other hand, Davies (1949) believes that these changes in electrolytes are due to renal causes. In Case 1 low serum chlorides were found, though no fall in blood pressure occurred during an attack. In Case 2, however, a profound state of peripheral circulatory failure developed, though no biochemical changes were revealed. Rawlings (1950) reported the case of a 22-year-old woman suffering from idiopathic porphyria who showed remarkable clinical improvement on becoming pregnant. The stimulus of pregnancy upon the anterior pituitary is well recognized, and Rawlings's report is also reminiscent of Hench's original observation on rheumatoid arthritis. It was felt, therefore, that a trial with A.C.T.H. was justified in Case 2.

The significance of the negative adrenaline test and the normal eosinophil response to A.C.T.H. is doubtful. According to Recant *et al.* (1950) adrenaline is a non-specific stimulator of the pituitary-adrenal axis. A positive test is said to suggest a normal response of the pituitary and adrenal glands, "but recent work has cast doubt on this interpretation" (Prunty *et al.*, 1951; Kark and Muehrcke, 1952). The normal response to A.C.T.H. in our case presumably indicates that the adrenals act in this respect normally, but our findings might suggest an anterior pituitary dysfunction. Further inference that acute porphyria is associated with endocrine changes might be drawn from the frequency with which attacks occur in the immediate premenstrual period, from the greater incidence of the disease among women, and, in our Case 2, from the patient's amenorrhoea, which has persisted for five months after the cessation of her symptoms of porphyria.

Clinically, the administration of parenteral fluid and A.C.T.H. undoubtedly improved Case 2 during her hypotensive phase. The effect of A.C.T.H. on porphyrin excretion cannot be judged on one case alone. The pronounced rise in urinary porphyrins on the third day of treatment followed by a lowered excretion during the period of A.C.T.H. treatment is of interest, but similar large fluctuations in daily porphyrin excretion have been recorded previously (Rimington, 1943); they render difficult the evaluation of any therapeutic trial. The mean daily uroporphyrin excretion during the periods immediately preceding and following the administration of A.C.T.H. were 19.6 and 14.4 mg. a day respectively. During treatment the mean figure was 31.4 mg. a day, or, if March 29 is excluded, 13.5 mg. a day. There is no indication, therefore, that the treatment with A.C.T.H. lowered the porphyrin excretion in this experiment. The drug may, however, be worthy of further trial in porphyria, especially in the so-called "adrenal insufficiency" phase of the disease, when this is present. Further experience by two of us (C. R. and A. G.) in the treatment of other cases of porphyria with A.C.T.H. leads us to conclude that the drug does not significantly alter the uroporphyrin excretion. In one case with persistently lowered serum chlorides (approximately 400 mg. per 100 ml., expressed as NaCl), unresponsive to added oral salt or parenteral normal saline, the administration of A.C.T.H. brought about a prompt and sustained restoration of the normal level (approximately 560 mg. per 100 ml.).

The presence of a toxic psychosis was considered as a possible contraindication to A.C.T.H. treatment. Towards the end of the week's course the patient became rather euphoric and excitable, and it was perhaps fortunate in this respect that A.C.T.H. was discontinued after seven days.

Sedation and the relief of pain in this condition present important and difficult problems. In both our cases opiates were used in large doses without any resultant addiction.

The prognosis is generally regarded as poor. Nesbitt (1944) remarks that the immediate mortality may be as high as 80 to 90% in acute episodes showing neurological manifestations. Happily, both our patients are now in good health. Case 1 is carrying on a useful job, and has recently been married. Case 2 is free from pain, is putting on weight, and has almost recovered her previous mental stability, five months after the cessation of her symptoms.

Summary

Two cases of acute idiopathic porphyria are described. One of them was treated with A.C.T.H. for a period of one week, but without any significant lowering of the mean daily uroporphyrin excretion.

The clinical manifestations and certain factors in diagnosis and treatment are discussed.

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The Effect of Certain Barbiturates on the Porphyrin Metabolism of Rabbits

By A. GOLDBERG
Nuffield Unit for the Investigation of Pyrrole Pigment Metabolism, Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

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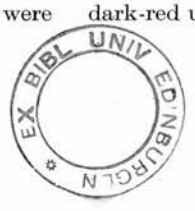
Since the introduction of the barbiturates to clinical medicine in 1903, there have been conflicting reports on their possible relation to human acute porphyria. Dobrschansky (1906) described a typical case of acute porphyria, without paralysis, occurring in a patient after prolonged administration of diethylbarbituric acid. Haxthausen (1927) reported the development of skin photosensitivity and the presence of excessive amounts of porphyrins in the urine of an epileptic who had been taking ethylphenylbarbiturate for some time. Eliaser & Kondo (1942), Denny-Brown & Sciarra (1945), Prunty (1946) and Jørgensen & With (1947) have described deterioration in the clinical state of acute porphyria patients with the development of severe or even fatal paralysis, after the administration of certain barbiturates, among which were ethylphenylbarbituric acid and ethyl(1-methylbutyl)barbituric acid. Waldenström (1939, 1940) was convinced that barbiturates may precipitate attacks in cases of latent porphyria and that they seriously affect the prognosis of the disease. On the other hand, Günther (1922), Turner (1938) and Discombe & D'Silva (1945) failed to note any relation between barbiturates and acute porphyria.

Information on the relation of barbiturates to normal human porphyrin metabolism has been obtained from observations on the urinary porphyrin excretion in cases of barbiturate poisoning. Rosendorff (1910), Sowden (1910) and MacLean (1912) found no abnormal porphyrinuria in patients who had ingested single large doses of diethylbarbituric acid, while Rommel (1912) observed dark-red urine in a patient who had taken 25 g. of



the same drug. Lehmann & Zinn (1910) have described a urinary pigmentation, suggestive of porphyrin and porphobilin, in a patient who had received 0·5 g. diethylbarbituric acid. They gave this drug in prolonged dosage to one rabbit without the production of similar pigments. Animal experiments were also carried out by Laubender & Monden (1938), who investigated the effects of diethyl, bromallyl*iso*propyl and ethyl*cyclo*hexenylbarbituric acids on the urinary porphyrins of rabbits. They failed to find any such effect in all these experiments with the exception of one animal treated with diethylbarbituric acid, which showed a slight rise of urinary porphyrins.

The barbiturates comprise a group of chemically related drugs, while acute porphyria is a rare disease. Moreover, it is usually impossible to tell from published records whether the barbiturates had precipitated the overt disease in a case of latent porphyria or had in fact provoked acute porphyria in a formerly healthy subject. To overcome this difficulty and the uncertainty in assessment of these clinical impressions, the effects of different barbiturates on the porphyrin metabolism of rabbits were studied. Out of nine barbiturates so tested, six had a significant effect on the urinary porphyrin excretion; those containing one or more allyl groups were particularly effective.

METHODS

Seventeen rabbits (average wt. 2·8 kg.) were housed in individual metabolism cages allowing the separation of urine and faeces. A preliminary base-line period of 4–7 days before drug administration was allowed during which the total daily urinary coproporphyrin and sometimes also faecal porphyrins were determined. If the animal died during an experiment, tissue porphyrins and porphobilinogen were also determined. The average duration of each course of barbiturate was 12 days. The types of barbiturates and their route of administration are recorded in Table 1.

Table 1. *Effect of barbiturates on the urinary excretion of coproporphyrin in rabbits*

The sodium barbiturates were given in equally divided dosage twice daily. A letter after a rabbit number signifies that the rabbit obtained the corresponding barbiturate on a primary course *a*, or on subsequent courses *b*, *c*, etc. Doses were administered by intramuscular injection unless indicated otherwise.

		Dosage		Mean urinary coproporphyrin	
Drug	Rabbit no.	Average level (mg./kg./day)	Duration (days)	Pre-dosage level (μg./day)	Increase (μg./day)
5:5-Diallylbarbituric acid	2*a*	113*	15	19·7	+193·7
	1*b*	103*	35	7·5	+169·5
	3	100	17	5·2	+119·2
	4*a*	128*	12	8·7	+88·3
	5	101·6	7	8	+86
	6	125	9	5·3	+84
	7	114	7	2	+51
	8	148	7	3·3	+47
Sodium 5-allyl-5-(1-methylbutyl)-barbiturate	9	172	7	3·7	+42·6
	10	115	12	3·8	+22·9
	11*b*	100	12	9·8	+20·2
	2*d*	82·8	12	19·7	+17·3
5-Allyl-5-*iso*propylbarbituric acid	2*b*	67·8†	12	19·7	+81·6
	12	76†	13	7	+40
	13	74†	12	7	+27
Sodium 5:5-diethylbarbiturate	14*b*	171	13	5	+ 9·7
	13*d*	140	12	7	+ 9·6
	15	169	13	1·8	+ 8·7
	16	144	12	2·4	+ 5·6
Sodium 5-ethyl-5-(1-methylbutyl)-barbiturate	11*a*	98·5	12	9·8	+15·2
	2*c*	110·5	12	19·7	+ 7·3
	4*b*	128	14	8·7	+ 2·3
Sodium 5-ethyl-5-phenylbarbiturate	14*a*	71	9	5	+14·7
	1*a*	67	20	7·5	+13·5
	13*c*	81	12	7	+11·7
	16*b*	82	12	2·4	+ 6·8
Sodium 5-*iso*amyl-5-ethylbarbiturate	13*b*	90	12	7	− 2
	2*e*	83	12	19·7	− 5·2
Sodium 5-butyl-5-ethylbarbiturate	17*a*	109·5	12	11·5	+ 5·5
	16*a*	98	12	2·4	+ 2·3
Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate	17*b*	218	12	11·5	+ 5·2
	2*f*	164	12	19·7	− 1·7

* Some doses given by gastric intubation.

† Given by gastric intubation.

If the rabbit survived this course, analyses were continued during a recovery period of 4–7 days. After such an interval, some rabbits were started on a different barbiturate as indicated in Table 1. The maximum sub-lethal dose of each drug was employed.

Quantitative analyses

Urinary porphyrins. These were determined by the method of Rimington & Sveinsson (1950) with the following modifications. Ether-soluble porphyrins were extracted by ether: acetic acid (10:1 by vol.) and after the ether had been washed three times with water, were transferred directly into 1·4 N-HCl for spectrophotometric determination (Beckman, model DU). One part of urine containing porphobilinogen, was diluted with 4 parts 2 N sodium acetate buffer pH 4·22, and placed in boiling water for 20 min. Appropriate dilution for spectrophotometric determination of the total uroporphyrin was made with HCl so that the final concentration of acid was 0·5 N.

Faecal coproporphyrin and tissue copro- and proto-porphyrin. Faeces and tissues were ground thoroughly in a mortar. Liver tissue was in addition comminuted in a Waring Blendor. The ether-soluble porphyrins from weighed portions of faeces and tissue were extracted by repeated shaking with ether: acetic acid (10:1, v/v) until the supernatant after centrifuging showed no porphyrin fluorescence. The porphyrins were then analysed as in the case of urine.

Porphobilinogen. Porphobilinogen in urine was determined by the method of Vahlquist (1939). Tissue dispersions were treated with about an equal volume of 20% (w/v) trichloroacetic acid, the mixture was centrifuged and the supernatant analysed as in the case of urine.

Blood examination. Haemoglobin was determined by the method of Rimington (1942). Erythrocyte counts and reticulocyte counts were carried out using standard techniques.

Urinary amino acids. These were examined by methods of Datta, Dent & Harris (1950).

Identification of porphyrins

The urines from rabbits treated with sodium allyl(1-methylbutyl)barbiturate or allyl*iso*propylbarbituric acid contained increased amounts of porphyrins, all of which were ether soluble. These urines were preserved with toluene and stored at 3°. The porphyrins were extracted with ether: acetic acid and, after the ether had been washed 3 times with water, were transferred into a minimal volume of 2·8 N-HCl and then precipitated at the isoelectric point (pH 3·1). The precipitate was centrifuged, dried, and esterified by contact with methanolic HCl for 24 hr. The porphyrin esters were transferred into $CHCl_3$ by the addition of saturated sodium acetate, the $CHCl_3$ layer washed once with water, once with dilute ammonia, and twice with water, in that order. The α band (absorption band at longest wavelength) of the porphyrin ester at this stage was noted on the Hartridge Reversion Spectroscope. A portion of the ester was dried, hydrolysed with 7 N-HCl for 36 hr. and chromatographed on paper (Nicholas & Rimington, 1951). The remainder was crystallized from $CHCl_3$: methanol (1:5, v/v). Melting points, which were not corrected, were determined on an electrically heated micro apparatus (A. Gallenkamp and Co. Ltd.). Isomer analysis was done by the method of Chu, Green & Chu (1951).

Urines from some rabbits treated with diallylbarbituric acid contained uroporphyrin and porphobilinogen as well as an increase in ether-soluble porphyrins. These combined urines were brought to pH 3–4 with glacial acetic acid and sufficient talc was added to adsorb all porphyrins. The talc was filtered, dried, and repeatedly eluted with 2 N ammonia until no porphyrin fluorescence appeared in the supernatant. The total alkaline eluates were then brought to the isoelectric point and the porphyrins thus precipitated were dried, esterified and taken into $CHCl_3$ as described above. The porphyrin esters were fractionated by chromatography on columns of aluminum oxide Grade IV and magnesium oxide Grade III (Nicholas, 1951). The fractions were defined by α band estimations and, after hydrolysis, by paper chromatography (Nicholas & Rimington, 1951). Isomer analysis of octacarboxylic porphyrin esters was performed by the method of Falk & Benson (1953), while that on tetra- and penta-carboxylic porphyrins was done by the method of Chu *et al.* (1951). The uroporphyrin ester was crystallized from chloroform: methanol.

RESULTS

Those barbiturates which produced the greatest increase of urinary porphyrins also caused the most prolonged hypnosis. Diallylbarbituric acid was the most effective drug, while allyl*iso*propylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, caused a higher excretion of urinary coproporphyrin and a more prolonged hypnosis than the remaining barbiturates. In each of three rabbits treated with diallylbarbituric acid, the drug was given sometimes by gastric intubation and sometimes by intramuscular injection. No significant quantitative difference of urinary porphyrin excretion was obtained by thus changing the route.

Urinary coproporphyrin. Table 1 records the average daily excretion of coproporphyrin in the urine during the pre-dosage period and the average deviation from this figure during administration of the drug. Coefficients of variation are not included since in almost every instance the results were quite obviously significant. Where an effective barbiturate was used, the urine coproporphyrin excretion showed a prompt increase after the 1st or 2nd day, rose to a maximum level on about the 6–7th day, remained at about that level during the subsequent administration of the drug and fell to normal within 2–3 days of its cessation.

Urinary uroporphyrin and porphobilinogen. In three rabbits (1*b*, 2*a* and 3) treated with diallylbarbituric acid, uroporphyrin and porphobilinogen appeared in the urine on the 7th, 7th and 8th days, respectively, and generally continued to be excreted for as long as the drug was given (Table 2 and Fig. 1).

Faecal coproporphyrin. In rabbit 1*b* the faecal coproporphyrin was determined before and during administration of diallylbarbituric acid. This showed that the faecal coproporphyrin rose at the same rate as the urine coproporphyrin from a

previous normal daily average of 38 μg./day to a daily average of 1495 μg./day.

Tissue porphyrins. Table 3 summarizes the tissue analyses for porphyrin of four normal rabbits, and five rabbits which had died from barbiturate intoxication at a stage when they were excreting increased urinary coproporphyrin. There was an increase of copro- and proto-porphyrin concentrations in the bile and liver in intoxicated rabbits, but the levels in the bone marrow showed no abnormality. The liver of rabbit 10 gave a faint but definitely positive reaction for porphobilinogen. Uroporphyrin was never found in any tissue.

Blood examination. Haemoglobin determinations, erythrocyte counts and reticulocyte counts were made immediately before, during and after administration of the drug to three rabbits treated with diallylbarbituric acid and one treated with allyl*iso*propyl barbituric acid. No significant change was observed in any of the erythrocyte counts or reticulocyte counts. In two of these rabbits (2*b* and 3), a slight fall (about 2 g. %) in the haemoglobin level was observed, while two rabbits (1*b* and 7) showed no significant change.

Table 2. *Daily urinary uroporphyrin and porphobilinogen excretion in rabbits receiving diallylbarbituric acid*

	Total uroporphyrin (μg./day)		Porphobilinogen (units*/day)	
Rabbit no.	Average	Maximum	Average	Maximum
1*b*	1720	4750	456	1607
2*a*	360	800	78	161
3	250	340	78	109

* Vahlquist (1939).

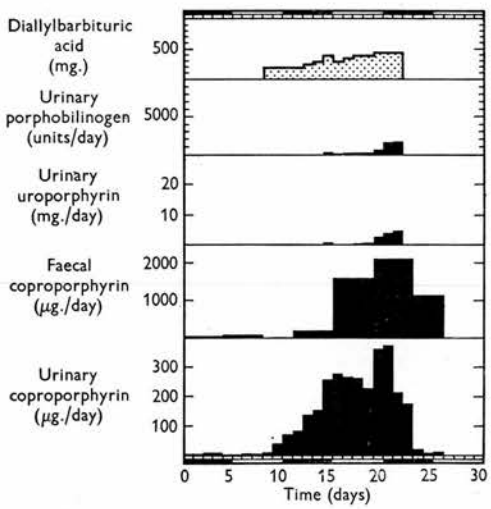


Fig. 1. Effects of diallylbarbituric acid on the urinary uroporphyrin, coproporphyrin and porphobilinogen and faecal coproporphyrin excretion of rabbit 1*b*.

Table 3. *Tissue porphyrin determinations on four normal rabbits and five rabbits treated with barbiturates*

The five rabbits died while excreting excessive urinary coproporphyrin. Figures represent μg./ml. (bile) or μg./g. (liver and marrow).

		Bile		Liver				Bone marrow			
	Rabbit no.	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin	Porpho-bilinogen	Uro-porphyrin	Copro-porphyrin	Proto-porphyrin	Porpho-bilinogen	Uro-porphyrin
Normal											
	Control I	0·15	Nil	0·10	0·10	Nil	Nil	0·10	Nil	Nil	Nil
	Control II	0·38	0·34	0·18	0·12	Nil	Nil	0·20	0·15	Nil	Nil
	Control III	0·74	0·41	0·18	0·51	Nil	Nil	0·22	0·24	Nil	Nil
	Control IV	Insufficient obtained		0·02	0·23	Nil	Nil	0·18	0·16	Nil	Nil
Diallylbarbituric acid											
	8	Insufficient obtained		5·5	49	Nil	Nil	0·10	0·12	Nil	Nil
	7	25	172	11·8	30·9	Nil	Nil	0·12	0·14	Nil	Nil
	5	5·2	84·0	3·2	4·66	Nil	Nil	0·15	0·22	Nil	Nil
Sodium allyl(1-methylbutyl)barbiturate											
	10	0·78	12·68	4·76	17	Faint +	Nil	0·10	0·24	Nil	Nil
	9	Insufficient obtained		1·91	3·84	Nil	Nil	0·30	0·31	Nil	Nil

Table 4. *Identification of urinary porphyrins. Summary of results*

	Free porphyrin	Porphyrin ester				
	Paper chromatography (Nicholas & Rimington, 1951)	Paper chromatography (*a*) Chu *et al.* 1951 α Band	(*b*) Falk & Benson, 1953	M.p. of crystals (°)	Summary
Diallylbarbituric acid	8 COOH	625·1	—	Mainly series III	274	Uroporphyrin III
	6 COOH	623·6	—	—	—	Hexacarboxylic porphyrin
	5 COOH	622·5	Series III	—	—	Pentacarboxylic porphyrin III
	4 COOH	621·1	Series III	—	—	Coproporphyrin III
Sodium allyl(1-methylbutyl)barbiturate	4 COOH	621·3	Mainly series III	—	154, remelt. 172–4	Coproporphyrin III
Allyl*iso*propylbarbituric acid	4 COOH	621·7	Mainly series III	—	150, remelt. 160	Coproporphyrin III

Urinary amino acids. In seven rabbits, in which a rise of urinary coproporphyrin was obtained, the urinary amino acid pattern was no different before and at the end of barbiturate administration.

Identification of porphyrins. The ether-soluble porphyrins obtained from the urines of rabbits treated with allyl*iso*propylbarbituric acid or sodium allyl(1-methylbutyl)barbiturate consisted entirely of coproporphyrin III. The ester m.p.'s and the results of paper chromatograms are shown in Table 4.

The porphyrin esters obtained from the urine of rabbits treated with diallylbarbituric acid were fractionated on a magnesium oxide column. This separated first an octacarboxylic porphyrin with an α band 625·0 mμ.; then two intermediate fractions with α bands 623·0 and 621·6 mμ., respectively; and finally a tetracarboxylic porphyrin with α band 621·1 mμ. The first fraction was rechromatographed on a magnesium oxide column and yielded a single porphyrin zone. The appearance of the crystals obtained from this zone was suggestive of uroporphyrin III. The m.p. (274°) and paper chromatography (Falk & Benson, 1953) showed the series III isomer to be that mainly present (see Table 4). The two intermediate fractions were further chromatographed on an aluminium oxide column. This allowed the separation of a main portion with α band 622·5 mμ. and a lesser portion with α band 623·5 mμ., which behaved as pentacarboxylic and hexacarboxylic porphyrins, respectively, when examined by paper chromatography (Nicholas & Rimington, 1951). On a paper chromatogram (Chu *et al.* 1951), the pentacarboxylic porphyrin had about the same mobility as coproporphyrin III. The final fraction was identified as coproporphyrin III by melting point and paper chromatography (see Table 4).

DISCUSSION

This work has shown that of the nine different barbiturates examined, six were capable of producing some effect on the porphyrin metabolism of rabbits. Of seventeen rabbits used, eight were given more than one barbiturate in separate courses. This was considered as a possible factor influencing results. However, another barbiturate was never started until the urinary coproporphyrin level had returned to its normal from the previous course, and the results in Table 1 show that the same drug, used on different animals, whether it was their first or subsequent barbiturate, generally gave corresponding results in porphyrin excretion. For the more effective barbiturates, sufficient numbers of rabbits were used, on a primary or subsequent barbiturate course, to demonstrate the unimportance of previous intoxication. Difference in effect upon porphyrin excretion must depend, among other things, upon the chemical constitution of the drug, the daily dosage employed and its duration, and also, possibly, upon the normal level of porphyrin excretion of the individual animal.

The results in Table 1 show that the barbiturates used may be classified in four groups according to their effectiveness (Fig. 2).

(1) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin in each of eight rabbits and, in three of these, the excretion also of uroporphyrin and porphobilinogen.

(2) Allyl*iso*propylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin.

(3) Sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate, which caused only a slight increase of coproporphyrin in the urine.

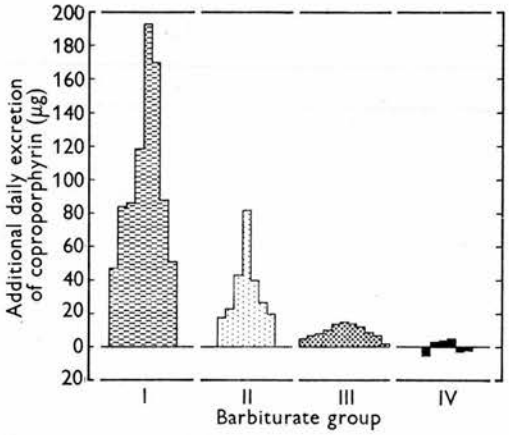


Fig. 2. Each vertical segment represents the average daily coproporphyrin output in a single rabbit during barbiturate administration in excess of (or less than) the normal average daily coproporphyrin excretion. I, diallylbarbituric acid; II, allyl*iso*propylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate. III, sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate. IV, sodium ethyl(1-methylbutyl)thiobarbiturate, sodium *iso*amylethylbarbiturate, and sodium butylethylbarbiturate.

(4) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium *iso*amylethylbarbiturate, and sodium butylethylbarbiturate, which did not alter significantly the urinary coproporphyrin excretion.

The possession of the allyl group thus appeared to make the barbiturate more effective in producing a rise of porphyrin in the urine. These drugs also caused deeper and more prolonged hypnosis in rabbits. Drugs possessing an allyl group have been known to give rise to pathological and metabolic changes in humans and in experimental animals. Popper (1936) and Jürgens (1951) have shown that allyl formate and *N*-(diallylacetyl)urea, respectively give rise to hepatic cellular degeneration in experimental animals. *N*-(Allyl*iso*propylacetyl)urea may cause thrombocytopenic purpura in humans (Ackroyd, 1949), may influence the course of human acute porphyria (Duesberg, 1932), and gives rise to the hepatic type of experimental porphyria in rabbits with the excretion of large amounts of uroporphyrin and porphobilinogen (Schmid & Schwartz, 1952). Diallylbarbituric acid has for some time been known to be one of the most toxic of the barbiturates and for this reason has generally been abandoned as an hypnotic in humans.

The finding in post-mortem tissue analysis that the liver and not the bone marrow contained excessive copro- and proto-porphyrin (and in one instance porphobilinogen) recalls the finding in the liver of porphobilinogen (Prunty, 1945) and uroporphyrin (Watson, Schwartz & Hawkinson, 1945) in human acute porphyria and of these two substances in experimental porphyria in rabbits (Schmid & Schwartz, 1952). It also may be related to the known hepatotoxic effect of other allyl derivatives in experimental animals. It is of interest, too, that the coproporphyrin and uroporphyrin produced in excess were the series III isomers. Rimington (1952) has proposed a scheme of possible enzymic derangements, occurring in the porphyrias and the toxic porphyrinurias. It is not possible from the present results to interpret the precise mechanism occurring in the barbiturate porphyrinurias, but a reasonable inference from them might be that some of the barbiturates, especially those containing the allyl group, may interfere with the enzymes which decarboxylate uroporphyrin III to coproporphyrin III and protoporphyrin and, perhaps, with those responsible for the transformation of protoporphyrin to haem. The main site of this interference may possibly be the liver.

The present findings raise the questions of the bearing of these animal experiments upon barbiturate medication in human beings and of the relation, if any, which exists between human acute porphyria and the disturbances of pigment metabolism caused by barbiturates in rabbits. The doses of barbiturates used in the animal experiments were proportionately (weight for weight) greatly in excess of those normally taken by humans. The rabbits, however, generally tolerated these daily doses well and were mostly able to survive the period of drug administration. Humans if given a proportionate amount of barbiturate almost certainly could not survive. A weight-for-weight comparison of the effect of the barbiturates on human and rabbit porphyrin metabolism would therefore be misleading. From these experiments, we may possibly infer that barbiturates in proportionately smaller dosage may affect the abnormally sensitive porphyrin metabolism in human acute porphyrias; the clinical impression that this does in fact take place may receive some substantiation from these results. The differing degree of effectiveness of the barbiturates in the experimental animal may also explain the divergence of opinion on this clinical impression, which was based on the observation of different barbiturates. It is of great interest that diallylbarbituric acid administered to a normal animal causes the excretion of porphobilinogen and uroporphyrin.

A further point of inference may be important. Barbiturates have been shown to inhibit some of the oxidative processes of brain tissue (Quastel & Wheatley, 1933), the degree of inhibition being directly proportional to the hypnotic activity of the drugs. These authors also emphasized the importance of the allyl group in this connexion. Changes in

nerve and brain tissue occurring in acute porphyria (Mason, Courville & Ziskind, 1933; Denny-Brown & Sciarra, 1945) may be the most important lesions responsible for the clinical results of this disease. It is possible that barbiturates might aggravate this upset in brain and nerve metabolism, as distinct from their effect on porphyrin metabolism. This might explain why some barbiturates, producing no marked effect on the porphyrin metabolism of rabbits, have been among those suspected of adversely affecting the course of acute porphyria.

SUMMARY

1. Barbiturates have been suspected of adversely affecting the clinical course of acute porphyria. To assess this clinical impression objectively, the effect of nine barbiturates on the porphyrin metabolism of rabbits has been observed.

2. These barbiturates may be classified into four groups. (i) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin III in each of eight experimental rabbits and in three of these the excretion also of uroporphyrin III and porphobilinogen. (ii) Allyl*iso*propylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin III. (iii) Sodium diethylbarbiturate, sodium ethyl-(1-methylbutyl)barbiturate and sodium ethylphenylbarbiturate, which caused only a slight coproporphyrin increase in the urine. (iv) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium *iso*amylethylbarbiturate and sodium butylethylbarbiturate, which did not alter significantly the urinary coproporphyrin excretion.

3. The effect of the presence of an allyl group in the barbiturate is discussed.

4. The significance of these results in animals is considered in relation to human acute porphyria.

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Experimentally produced porphyria in animals

By A. Goldberg and C. Rimington, F.R.S.

*The Nuffield Unit for the Investigation of Pyrrole Pigment Metabolism, Department of Chemical Pathology, University College Hospital Medical School, London, W.C.*1

With notes on histopathological studies

By J. C. B. Fenton

Department of Pathology, University College Hospital Medical School

(*Received* 20 *May* 1954)

[Plates 10 and 11]

The chemical findings of Schmid & Schwartz (1952) in experimental porphyria of rabbits induced by sedormid have been confirmed.

Since sedormid is hypnotic, a group of related drugs has been tested to find one which might produce the chemical picture in animals without hypnosis. Such a drug is allyl-*iso*propyl-acetamide (A.I.A.). In this investigation, the constant chemical structure affecting porphyrin metabolism was found to be $CH_2{=}CH{-}CH_2{-}CHR{-}CO{-}NH{-}$. Some rabbits excrete large amounts of porphobilinogen and uroporphyrin when given either sedormid or A.I.A., others produce little. It is suggested that the cause of this difference is related to a variability of the individual rabbit liver to deal effectively with these drugs.

Rabbits, intoxicated with either drug, became constipated, had poor appetite and lost weight. They did not become paralyzed, nor show any change in systolic blood pressure or in their haematological values. Two fowls, one also given a barbiturate, and nine rats were intoxicated with allyl-*iso*propyl-acetamide. Although these animals excreted relatively high levels of porphobilinogen and porphyrins, they did not develop paralysis.

The experimentally induced porphyria in animals is compared with human acute porphyria. The effects are described of reticulo-endothelial blockade, splenectomy and barbiturate administration on porphyria induced experimentally in rabbits.

Experimental porphyria appears to be due to an overproduction of porphyrins, rather than to an under-utilization of porphyrin pigments.

An atypical porphobilinogen reaction is described. It is present in the early stage of drug intoxication in rabbits and has also been noted in human acute porphyria at low levels of porphobilinogen excretion.

Introduction

Schmid & Schwartz (1952) have described an experimental porphyria in rabbits induced by sedormid (allyl-*iso*propyl-acetylurea) in which they noted the urinary excretion of large amounts of uroporphyrin and porphobilinogen and in many of

their animals the development of 'transient paralysis of the hind legs and bladder and functional gastrointestinal disturbances', reminiscent of human acute porphyria. We have confirmed the chemical findings of these authors, but the difficulty in making a clinical assessment of rabbits treated with sedormid, which is a profound hypnotic, has led us to investigate related drugs, one of which might produce the same chemical features, but yet be *non-hypnotic*. Such a drug is *allyl*-iso*propyl*-*acetamide* (Goldberg 1953) (henceforward called A.I.A.). This drug has given a clearer view of what happens to rabbits affected by a pigment dyscrasia, apparently the same as in human acute porphyria.

Comparison of the effects of these related drugs on the porphyrin metabolism of rabbits, together with a previously reported similar study of certain barbiturates (Goldberg 1954), has also allowed the definition of a chemical structure which may interfere with normal porphyrin formation. Some rabbits receiving sedormid or A.I.A. excreted large amounts of porphobilinogen and uroporphyrin, while others excreted only small amounts of these materials. A study of this difference in individual animals and its relation to corresponding differences in the pathological histology of the liver and urinary amino-acid excretion has confirmed the importance of the liver in porphyrin metabolism (Prunty 1945; Watson, Schwartz & Hawkinson 1945).

The occasionally beneficial effect of splenectomy in congenital porphyria (Aldrich, Hawkinson, Grinstein & Watson 1951) suggested a trial of the effect on a rabbit of splenectomy between two separate courses of sedormid.

The type of cells in which porphyrins are normally fabricated is unknown, but the liver is probably of great importance. An attempt has been made to investigate the role of the reticulo-endothelial cells of the liver in the porphyrin metabolism of experimental porphyria by means of the R.E. blockading substance thorium dioxide (Gottlieb 1934), although it is doubtful if a complete blockade can be achieved.

For some time the barbiturates have been considered to have an adverse effect on patients with acute porphyria. Waldenström (1939) considered that they might precipitate neurological manifestations in patients who might otherwise suffer only the abdominal symptoms of this disease. For this reason the effect was observed of a barbiturate given to an A.I.A.-intoxicated rabbit already excreting large quantities of porphobilinogen and porphyrins. Further to explore any possible relationship between experimental porphyria, paralysis and barbiturates, an experimental porphyria, with and without the addition of a barbiturate, was induced in fowls, animals known to be susceptible to metabolic derangement of their nervous system. An experimental porphyria has also been induced in rats by means of A.I.A.

Methods and materials

Rabbits (2 to 4 kg body weight) were kept in metabolism cages, allowing the separate collection of urine and faeces. In general, porphyrin and porphobilinogen determinations were carried out on 24 h collections of urines; in a few cases, 48 h collections were used. Where faecal porphyrins were determined, 2-, 3- or 4-day

specimens were pooled, having been collected daily and stored at 2° C. In most cases, a 4- to 7-day base-line period was allowed before the drug was given. Oral drugs were administered once daily by gastric intubation.

References to many of the methods used in this work have been described previously (Goldberg 1954). These include urinary, faecal and tissue porphyrin and porphobilinogen determinations. Uroporphyrin in tissues was determined, after extraction of ether-soluble porphyrins, by shaking repeatedly with N-ammonium hydroxide until extracts no longer exhibited red fluorescence when brought to 0·5N in HCl. The dissolved uroporphyrin was determined spectrophotometrically as in the case of urine. Identification of porphyrins by chromatographic methods, melting-point and spectrophotometric determinations and haematological measurements were carried out as by Goldberg (1954). Platelet counts were done by the direct method using Rees-Ecker solution (Wintrobe 1951).

The isolation of crystalline porphobilinogen and its identification by paper chromatography were carried out by the methods described by Westall (1952) or Cookson & Rimington (1954).

Blood pressure in the rabbits was determined by the method of Grant & Rothschild (1934). Four consecutive readings were taken under their recommended conditions at the same time each morning for several days before the drug was given and then similarly during the course of its administration.

All rabbits' urines were examined initially, and at the end of the drug course for amino-acids by the method of Datta, Dent & Harris (1950). Urines found to contain many amino-acids were then further examined by the method of Dent (1948).

The thorium dioxide used was in a 25% colloidal suspension ('thorotrast'—Heyden).

Results

Porphyrins

Urine. Table 1 summarizes the urinary uroporphyrin* and porphobilinogen excretion of twenty-three rabbits, intoxicated with allyl-*iso*propyl-acetamide (A.I.A.) (nine rabbits), sedormid (six rabbits), propyl-*iso*propyl-acetamide (three rabbits) and allyl-*iso*propyl-acetic acid (five rabbits). For comparison, the results of a similar series, previously reported (Goldberg 1954) using diallyl barbituric acid, have been added to the present group. A.I.A. and sedormid caused an immediate increase above normal in the level of urinary coproporphyrin, mounting rapidly stepwise with continued administration of the drug until the 4th to 7th day, when porphobilinogen and uroporphyrin were noted in the urine (see figures 1 and 2). From this point, the urinary coproporphyrin excretion remained approximately constant, but the porphobilinogen and uroporphyrin mounted to reach maximum levels 2 to 3 days later. These drugs were continued in every case except two (nos. 5

* Urines were heated with 2N acetate buffer pH 4·2 for 20 min at 100° C to convert porphobilinogen present into uroporphyrin and the total uroporphyrin then determined spectrophotometrically (see Methods). The term 'urinary uroporphyrin' is used throughout this paper to indicate the total uroporphyrin so measured.

and 12) until the rabbit died. In rabbits 5 and 12, withdrawal of the drug led to a rapid (within 48 h) cessation of urinary porphyrin and porphobilinogen excretion and a more gradual lessening of coproporphyrin excretion until the former normal levels were regained in 4 days' time. No significant difference was noted when comparing the pattern of porphyrin and porphobilinogen excretion of the sedormid

TABLE 1. EFFECTS OF DRUGS ON URINARY PORPHYRIN AND PORPHOBILINOGEN EXCRETION OF RABBITS

rabbit no.	route*	dose (mg/kg/day)	duration (days)	uroporphyrin (mg/day) mean	uroporphyrin (mg/day) max.	porphobilinogen (mg/day) mean	porphobilinogen (mg/day) max.	copro-porphyrin (μg/day) additional to normal
				A.I.A.				
1	I.G.	187	14	1·52	2·62	5·3	11·2	289
2	I.G.	226	11	0·43	1·48	2·7	11·0	264
3	I.G.	217	8	1·47	3·87	6·3	20·1	272
4	I.G.	170	13	10·21	18·33	53·7	101·8	292
5	I.G.	182	23	6·32	15·85	36·8	70·4	342
6	I.G.	176	8	7·37	12·52	19·8	34·5	203
7	I.G.	181	12	10·26	17·7	63·6	116·2	188
8	I.G.	183	5	2·46	7·66	18·81	62·4	217
9	I.G.	204	13	3·85	13·44	9·05	24·4	125
				sedormid				
10	I.G.	257	9	1·53	5·5	5·4	11·5	120
11	I.G.	230	9	6·6	19·0	7·4	18·2	182
12	I.G.	217	11	11·29	27·0	38·0	64·5	94
13	I.G.	187	8	8·8	15·2	not done		279
14	I.G.	212	14	1·78	3·8	3·7	9·0	104
15	I.G.	187	7	2·5	3·2	not done		126
				P.I.A.				
16	I.G.	236	6	—	—	—	—	nil
17	I.G.	256	12	—	—	—	—	5
18	I.G.	143	7	—	—	—	—	nil
				A.I.Ac. acid				
19	I.G.	372	5	—	—	—	—	5
20	I.G.	154	12	—	—	—	—	16
21	I.M.	193	6	—	—	—	—	10
22	I.M.	267	9	—	—	—	—	9
23	I.G.	128	9	—	—	—	—	2
				dial				
24	I.M. or I.G.	113	15	0·36	0·80	0·6	1·24	194
25	I.M. or I.G.	103	35	1·72	4·75	3·6	12·4	169
26	I.M.	100	17	0·25	0·34	0·6	0·84	119
27	I.M. or I.G.	128	12	—	—	—	—	88
28	I.M.	102	7	—	—	—	—	86
29	I.M.	125	9	—	—	—	—	84
30	I.M.	114	7	—	—	—	—	51
31	I.M.	148	7	—	—	—	—	47

* I.G. = intragastric; I.M. = intramuscular; A.I.A. = allyl-*iso*propyl-acetamide; P.I.A. = *n*-propyl-*iso*propyl-acetamide; A.I.Ac. acid = allyl-*iso*propyl-acetic acid; dial = diallyl-barbituric acid.

rabbits with that of the A.I.A. rabbits, except that one A.I.A.-intoxicated rabbit (no. 5) continued to excrete large amounts of these materials for 3 weeks, a duration not found possible with sedormid rabbits. Some rabbits (nos. 4, 5, 6, 7, 11 and 12) excreted large amounts of porphobilinogen and uroporphyrin and continued to do so for as long as the drug was given (nos. 5 and 12), or until some intercurrent cause of death intervened, e.g. bronchopneumonia in rabbits 7 and 12 or uraemia in

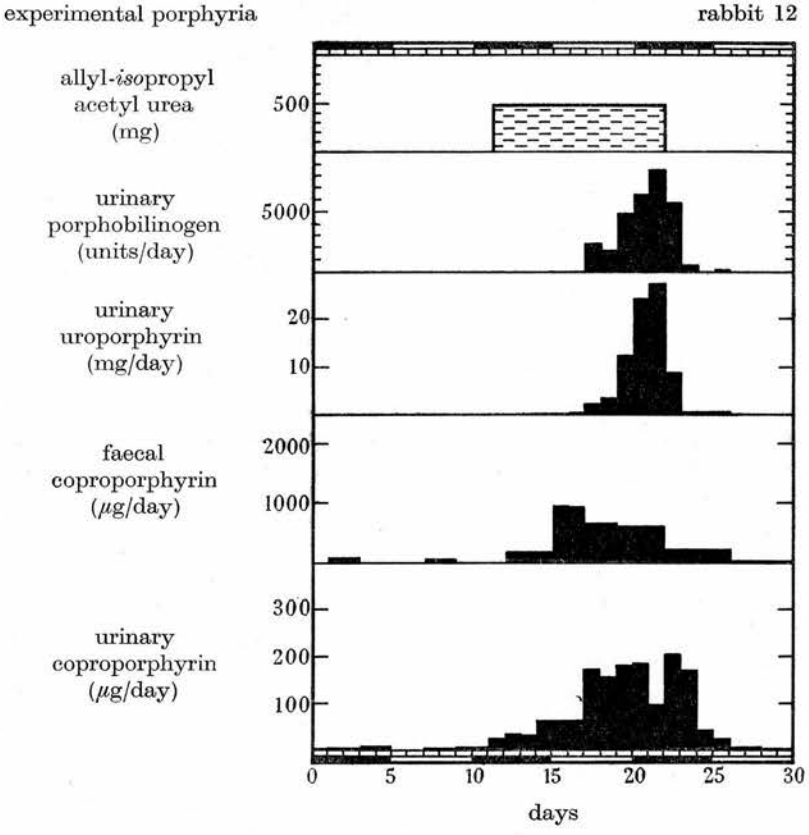


FIGURE 1. Effect of sedormid in a rabbit on its excretion of urinary porphobilinogen and coproporphyrin and faecal coproporphyrin. 130 units of porphobilinogen equal 1 mg of porphobilinogen (Westall 1952).

rabbit 4. Other rabbits (nos. 1, 2, 3 and 10) reached maximum excretions of uroporphyrin and porphobilinogen at much lower levels and ceased excreting these substances, although still maintaining a coproporphyrinuria, 1 or 2 days before death from severe hepatic damage.

The chemical identification of urinary porphyrins is summarized in table 2.

n-Propyl-*iso*propyl-acetamide showed no effect on the porphyrin excretion of rabbits, while allyl-*iso*propyl-acetic acid caused slight increases in coproporphyrin excretion above normal (more than 5μg/day) in three out of five rabbits tested.

Faeces. Faecal coproporphyrin was determined in six rabbits (nos. 5, 11, 12, 13, 14 and 15) whose daily excretion rose from normal (pre-drug) levels of 254, 44, 70,

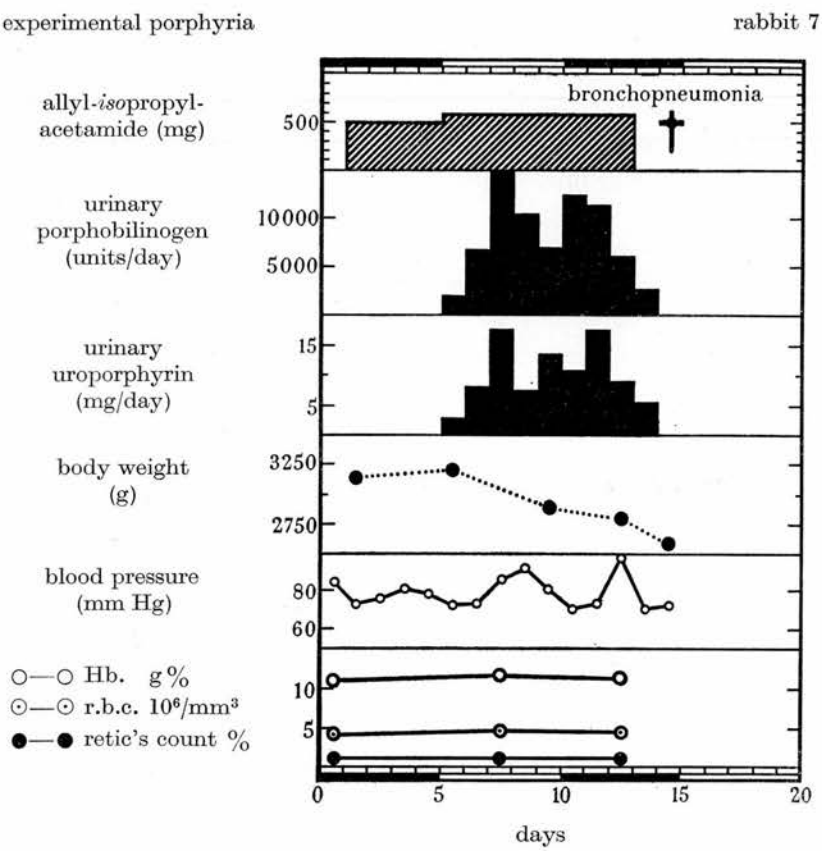


FIGURE 2. Effect of allyl-*iso*propyl-acetamide in a rabbit on its excretion of urinary porphobilinogen and on its body weight, systolic blood pressure and haematological values. 130 units of porphobilinogen equal 1 mg of porphobilinogen (Westall 1952).

TABLE 2. IDENTIFICATION OF PORPHYRINS. SUMMARY OF RESULTS

drug	material	free porphyrin no. of carboxyl groups	α band in $CHCl_3$ (mμ)	porphyrin methyl ester isomeric series by paper chromatography	m.p. of crystals (°C)	summary
sedormid	urine	8	625·3	mainly III	254–258	uroporphyrin III
		5	622·4	—	—	—
		4	621·2	III	—	coproporphyrin III
A.I.A.	urine	8	625·6	mainly III	247–261	uroporphyrin III
	plasma*	8	625·0	I and III	—	uroporphyrin III and I
	bile	8	—	III	—	uroporphyrin III
		4	—	III	—	coproporphyrin III
		2	630·2	—	—	protoporphyrin

* Ether-insoluble fraction only.

64, 6 and 61 μg to levels of 495, 315, 942, 473, 1060 and 221 μg respectively. In the two animals which survived (nos. 5 and 12), faecal coproporphyrin levels were determined after the cessation of the drug. In no. 5 the average daily coproporphyrin excretion for the first 8 days of this period was 3659 μg. This animal was severely constipated during the drug course, but excreted a normal quantity of faeces as soon as the drug was withdrawn. The large rise from 495 to 3659 μg/day was thus probably due to rapid evacuation of the overloaded bowel. In rabbit no. 12 constipation was less marked, and the faecal coproporphyrin fell from a mean of 602 μg/day on the last 3 days of the drug course to 202 μg/day on the 3 days following the cessation of dosage.

In the course of these determinations it became evident that the faecal protoporphyrin was considerably raised. The quantitative extraction of this porphyrin was rendered unsatisfactory by the presence of large amounts of chlorophyll-type pigments in the stool.

Bile. Increased coproporphyrin and protoporphyrin levels were found in the bile of all rabbits showing an increased urinary coproporphyrin excretion (see table 3). In six rabbits intoxicated with either A.I.A. or sedormid, an ether-insoluble porphyrin was obtained. On spectrophotometric examination in 0·5 N-HCl this porphyrin had a maximal absorption at 405 mμ. It was further investigated by chromatographic methods and shown to be uroporphyrin III. The coproporphyrin was identified by paper chromatographic methods as of the series III type (see table 2).

Plasma. In three rabbits (nos. 4, 7 and 8) the plasma or serum at post-mortem showed red fluorescence. In one of these (no. 4) this was found, by further investigation, to be due to coproporphyrin and uroporphyrin III with some uroporphyrin I (see table 2).

Tissues (see table 3). The coproporphyrin and protoporphyrin of liver and bile showed increases above normal, proportional to the degree of porphyrin increase in the urine. In A.I.A.- and sedormid-intoxicated rabbits, the spleen, marrow and kidney coproporphyrin and protoporphyrin were slightly raised above normal, but not to the same degree as in liver and bile. In rabbit 4, portions of skeletal muscle tissue showed marked porphyrin fluorescence. Two upper incisor teeth which had accidentally been broken at the beginning of the experiment grew to their previous length in 1 week. At post-mortem examination, the roots of these teeth had porphyrin fluorescence. Rabbit 16 (*n*-propyl-*iso*propyl-acetamide) had no porphyrin increase in any tissue. Rabbits 19, 21 and 22 (allyl-*iso*propyl-acetic acid) had small increases in liver and bile coproporphyrin and protoporphyrin, while marrow coproporphyrin and protoporphyrin were also slightly raised in rabbit 22.

Porphobilinogen

Urine. Porphobilinogen was isolated from rabbit urine by the method of Westall (1952) or that of Cookson & Rimington (1954) and found to be identical in all respects with porphobilinogen from acute porphyria urine.

Plasma. In four rabbits (nos. 1, 4, 7 and 8) the blood plasma tested qualitatively for porphobilinogen (Vahlquist 1939) at the height of the induced attack, or at

TABLE 3. SUMMARY OF TISSUE PORPHYRIN AND PORPHOBILINOGEN DETERMINATIONS

(The results are expressed as μg/g or ml. of tissue or bile respectively.)

	liver				bile*			spleen†		marrow‡		kidney			
rabbit no.	copro.	proto.	pbg.	uro.	copro.	proto.	uro.	copro.	proto.	copro.	proto.	copro.	proto.	pbg.	uro.
							controls								
A	0·10	0·10	nil	nil	0·15	nil	nil	0·15	0·10	0·10	nil	0·11	0·03	nil	nil
B	0·18	0·12	nil	nil	0·38	0·34	nil	insufficient		0·20	0·15	0·03	0·02	nil	nil
C	0·18	0·51	nil	nil	0·74	0·41	nil	insufficient		0·22	0·24	0·24	0·07	nil	nil
D	0·02	0·23	nil	nil		insufficient		0·08	0·16	0·18	0·16	0·10	0·03	nil	nil
							A.I.A.								
1	4·67	3·96	nil	nil		insufficient		2·61	1·41	1·14	0·74	4·67	3·96	nil	nil
2	3·94	17·48	nil	nil		insufficient		insufficient		0·16	0·43	—	—	—	—
3	0·52	1·60	nil	nil	69·5	165·00	152·00	0·24	0·24	1·31	0·69	—	—	—	—
4	5·50	32·30	36·0	2·58	4·9	12·00	10·00	0·90	1·70	0·90	2·00	0·50	0·47	+	2·20
6	2·75	50·00	+ + +	nil	159·0	231·00	22·50	0·60	1·15	0·13	0·19	3·24	1·31	+	2·29
7	4·62	73·00	nil	nil	115·0	224·00	nil	—	—	trace	trace	—	—	—	—
8	2·55	7·91	nil	85·00	51·0	308·00	97·00	0·54	nil	0·84	0·69	—	—	—	—
9	2·70	14·85	nil	nil	57·5	93·00	nil	0·44	0·56	0·12	0·17	—	—	—	—
							sedormid								
10	0·54	26·00	nil	nil	—	—	—	—	—	—	—	—	—	—	—
11	5·65	3·00	2·9	nil	—	—	—	—	—	—	—	—	—	—	—
12	8·00	12·80	6·0	nil	33·0	219·00	128·00	—	—	—	—	—	—	—	—
13	0·30	3·12	nil	nil	13·6	44·20	37·00	—	—	—	—	—	—	—	—
14	4·62	7·39	nil	nil	—	—	—	—	—	—	—	—	—	+	—
15	2·86	4·68	26·0	nil	—	—	—	—	—	nil	nil	1·30	1·10	nil	—
							P.I.A.								
16	0·21	0·26	nil	nil		insufficient		—	—	0·05	0·26	—	—	—	—
							A.I.Ac. acid								
19	0·45	0·52	nil	nil	1·1	1·99	nil	0·13	0·18	0·10	0·10	—	—	—	—
21	0·40	1·00	nil	nil	0·43	1·07	nil	—	—	0·16	0·14	—	—	—	—
22	0·53	2·82	nil	nil	1·18	1·73	nil	—	—	1·18	1·73	—	—	—	—

A.I.A. = allyl-*iso*propyl-acetamide; P.I.A. = *n*-propyl-*iso*propyl-acetamide; A.I.Ac. acid = allyl-*iso*propyl-acetic acid; copro. = coproporphyrin; uro. = uroporphyrin; proto. = protoporphyrin; pbg. = porphobilinogen.

* Bile porphobilinogen *nil* in every case.

† Spleen porphobilinogen a *trace* in no. 8, otherwise *nil*. Spleen uroporphyrin 1·84 in no. 4, otherwise *nil*.

‡ Marrow porphobilinogen a *trace* in no. 8, otherwise *nil*. Marrow uroporphyrin 0·7 in no. 4, otherwise *nil*.

post-mortem, gave a positive result. In the case of rabbit 8, a spectrophotometric extinction curve was also obtained showing maxima at 527 and 556 mμ, characteristic of the product of porphobilinogen and Ehrlich's aldehyde reagent. The concentration of plasma porphobilinogen in rabbit 4 post-mortem was 105 μg/ml. (for quantitative determination of porphobilinogen see Westall (1952)).

Tissues. In five rabbits there was a positive porphobilinogen reaction in the liver; in three of these it was also positive in the kidney. Faeces, brain, bile, spleen and marrow were negative for porphobilinogen, with the exception that in rabbit 8 traces of porphobilinogen were present in spleen and marrow. In this animal the plasma porphobilinogen was strongly positive, and these trace reactions could have been derived from the blood which its spleen and marrow contained.

Atypical porphobilinogen reaction

The urines of rabbits undergoing drug treatment were examined for porphobilinogen qualitatively according to Watson & Schwartz (1941) and quantitatively according to Vahlquist (1939). In the former method, Ehrlich's aldehyde reagent is followed by saturated sodium acetate; Vahlquist used a stronger reagent without acetate. Pure porphobilinogen in aqueous solution gives a red colour with both reagents, and there is no intensification, rather a diminution, of colour on addition of acetate. The urines of rabbits intoxicated by A.I.A. or sedormid gave a positive Watson & Schwartz test from about the 2nd or 3rd day of dosing. Addition of the Ehrlich reagent these authors used often led to no red colour at all, but on subsequent addition of sodium acetate quite intense red colours developed. The Vahlquist reaction only became positive some 2 to 4 days later. In neither case was the colour extractable by chloroform.

There appeared to be some material in the earlier urines which modified the course of the Ehrlich reaction, and we refer to these reactions as 'atypical porphobilinogen reactions'. The atypical reaction became less pronounced, and finally disappeared, as the strength of the Vahlquist reactions increased. Normal rabbit urines sometimes gave a faintly positive atypical reaction (Vahlquist reaction negative), and similar atypical reactions have been seen in some human urines containing low levels of porphobilinogen from cases of acute porphyria. Atypical reactions have not been observed in the urines of rats intoxicated by A.I.A.

Urinary amino-acids

These were identified in ten rabbits (A.I.A.- or sedormid-intoxicated) by means of paper chromatography. Normal rabbit urine (10 to 15 μl. in method of Datta *et al.* (1950) and 15 to 25 μl. in method of Dent (1948)) shows only a glycine spot. In rabbit 14 no abnormality was detected at the end of the drug course. In five rabbits, all high excretors of uroporphyrin and porphobilinogen (nos. 4, 5, 6, 7 and 12), the glycine spot at the end of the course was markedly reduced in intensity from that before the drug was given. In rabbits 4 and 6, this diminution was established by an approximately quantitative experiment. Volumes of urine, from before and at the end of the drug course, corresponding to the same fraction of the total daily urine output, were placed on to the same paper chromatogram and

developed by either phenol or butanol acetic acid respectively, in a one-dimensional run. The dilution of the stronger urine necessary to match the weaker was assessed and confirmed experimentally by further chromatographic runs. This experiment showed administration of the drug in both animals to have caused at least four-fold reduction in urinary excretion of glycine. Rabbits 1, 10 and 12 (all poor excretors of uroporphyrin and porphobilinogen) voided many amino-acids at the end of the drug course, the day before death, viz.:

Rabbit 1 excreted taurine, alanine, glutamic acid, glutamine, ethanolamine phosphate and glycine (see figure 3, plate 10).

Rabbit 10 excreted glutamic acid, glycine, α-amino butyric acid, lysine, threonine, taurine, small amounts of histidine, methyl histidine, arginine, serine and a trace of leucine (see figure 4, plate 10).

Rabbit 12 excreted glutamic acid, glycine, alanine, taurine, threonine, β-alanine, histidine, trace valine, trace tyrosine, leucine and some aspartic acid.

Clinical findings of A.I.A.- and sedormid-intoxicated rabbits

Neurological

Rabbits intoxicated with sedormid differed from those treated with A.I.A. Sedormid proved to be a profound hypnotic, the animals remaining unconscious for about 10 h after each dose of the drug. Those treated with A.I.A. never lost consciousness, but a few appeared slightly dazed for about 1 to 1·5 h after the administration of the drug. It was thus very difficult to evaluate the claim of Schmid & Schwartz (1952) that sedormid-treated rabbits exhibited paralysis or paresis. Using A.I.A. alone, limb paralyses or pareses never occurred, and these rabbits remained normally mobile and active even though excreting amounts of uroporphyrin as high as 7 mg/day or more for many days; rabbit no. 5 maintained an excretion near this level during an entire 3 weeks' course of the drug.

Gastro-intestinal

Appetite and weight. Both groups quickly developed a relative anorexia; they left most of their pellet (M.R.C. no. 18, Bruce & Parkes 1946) diet, but usually ate cabbage and drank sufficient water to maintain their normal urinary volume. The rabbits lost weight steadily; mean daily losses for those on A.I.A. being 52 g (six rabbits tested), and for those on sedormid 36 g (five rabbits tested). In the case of rabbit 5, normal appetite returned the day following cessation of the drug (A.I.A.), and the body weight gradually rose to its previous level.

Faeces. Rabbits of both groups became quickly constipated. Thus average daily faecal weights were recorded of 87 g before and 17 g during the administration of A.I.A., and of 60 g before and 25 g during the administration of sedormid. In most rabbits this drop took place the day following the first dose of the drug. The stool weight of rabbit 5 rose from an average level of 4 g/day during the period of drug administration to 89 g/day on the second day after withdrawal of the drug.

Straight X-ray of the abdomen was done on two rabbits before and at weekly intervals during A.I.A. administration. In one (no. 1) no difference was observed,

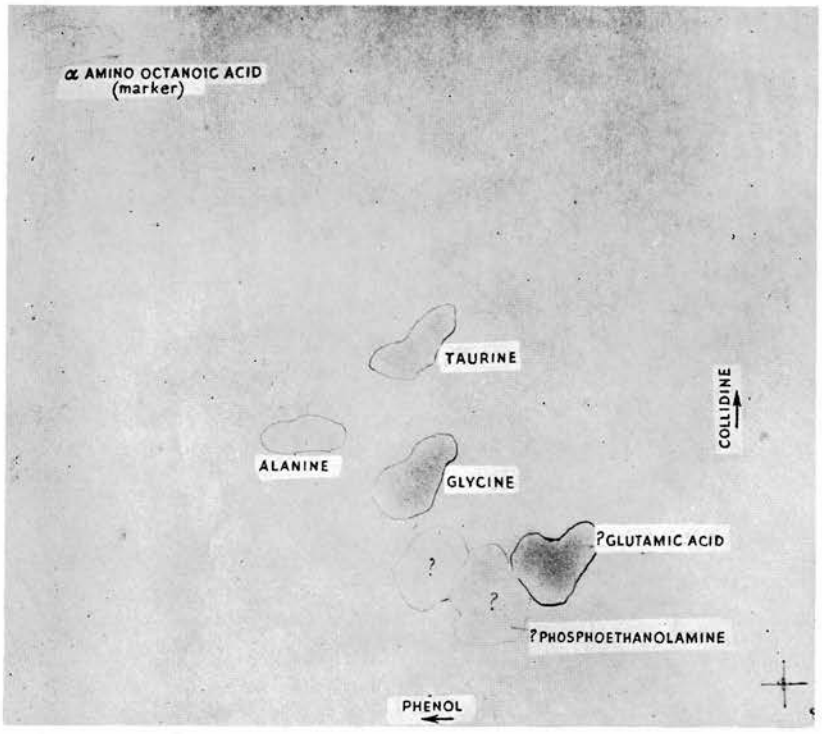


FIGURE 3. Urinary amino-acids of rabbit 1 on last day of experiment.

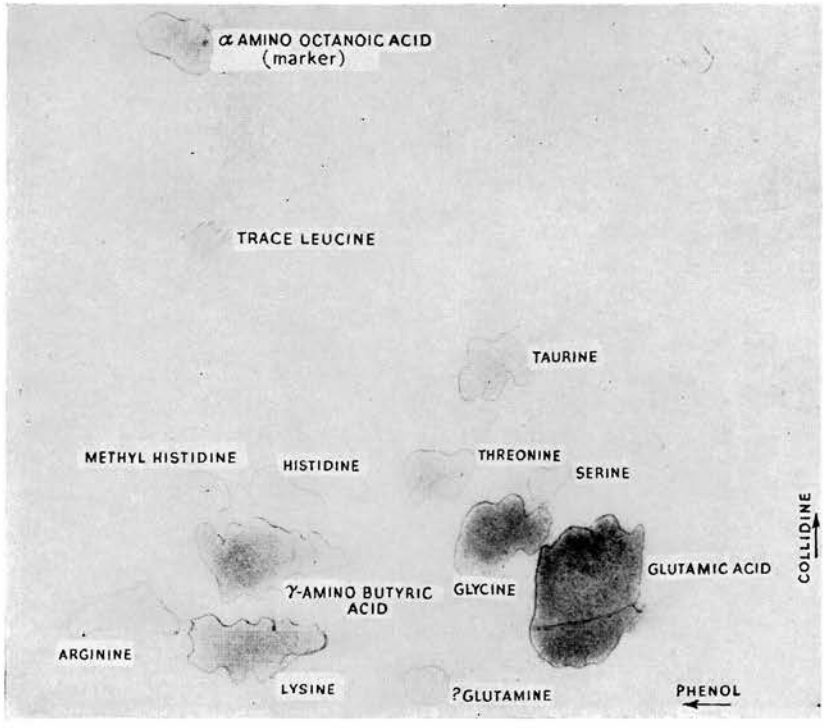


FIGURE 4. Urinary amino-acids of rabbit 10 on last day of experiment.

(*Facing p.* 266)

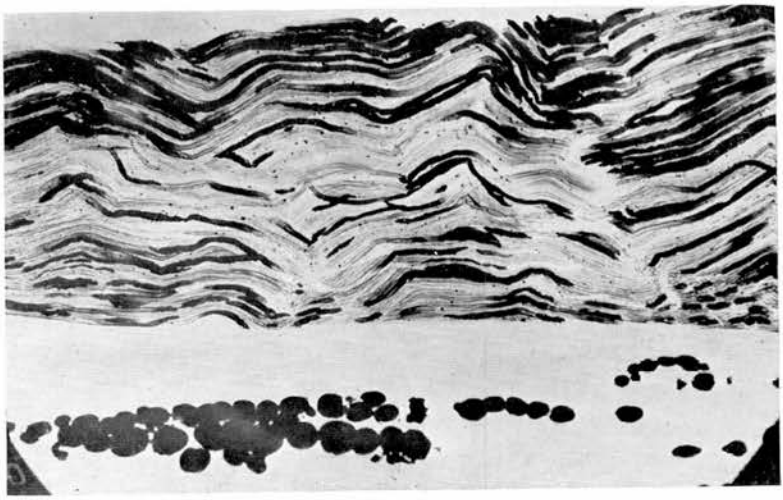


FIGURE 5. Sciatic nerve of rabbit 4 showing blackening of fibres by Marchi stain. (Magn. × 55.)

while in the other (no. 5), a progressively increasing gaseous distention of loops of bowel was revealed. This appearance gradually returned to normal on withdrawal of the drug.

Blood-pressure

Table 4 summarizes the results of systolic blood-pressure readings of four rabbits intoxicated with A.I.A. It is difficult to presume any significant change of blood pressure consequent on drug administration. The systolic blood pressure of rabbit 10 maintained a steady level, averaging 73 mm Hg in the first 6 days of its sedormid course, during which there was a mounting excretion of coproporphyrin and later of uroporphyrin and porphobilinogen. On the 7th, 8th, 9th and 10th days, the blood pressure fell to 38, 42, 40 and 14 mm Hg respectively, during which time there was a fall of porphobilinogen and uroporphyrin excretion, although the drug was maintained at the same dose. The rabbit died on the 10th day; post-mortem examination revealed hepatic necrosis.

TABLE 4. SYSTOLIC BLOOD PRESSURE OF RABBITS WITH EXPERIMENTAL PORPHYRIA

rabbit no.	mean uroporphyrin excretion (mg/day)	mean daily B.P. (systolic) (mm Hg)	
		before drug	during drug
1	1·52	78	85
3	1·47	64	69
5	6·32	81	74
7	10·26	83	78

Haematological

The results of serial haematological determinations in five rabbits are summarized in table 5, together with total loss of porphyrin in the urine during the course of the experiment. One animal (no. 5) provided data for porphyrin loss in both urine and faeces. The determinations of Hb, R.B.C. and reticulocytes showed no significant variation. Determinations of blood platelets and clot retraction on two rabbits before and during sedormic administration also showed no significant change.

Dermatological

The rabbits showed no evidence of photosensitivity although in a normally bright room. The sedormid skin patch test of Ackroyd (1949) was applied for 48 h to the shaved skin of the inferior dorsal area of rabbit 11, during its course of sedormid. There was no skin reaction to this test.

Effect of barbiturate

The effect was observed of a small dose of a barbiturate on a rabbit (no. 4; 3·3 kg) already excreting high levels of uroporphyrin and porphobilinogen under the influence of A.I.A. intoxication. 100 mg of sodium allyl-(1-methyl butyl)-barbiturate was injected intramuscularly on the 14th day of the drug course. The rabbit remained unconscious for the next 24 h. In normal rabbits the same dose

relative to body weight of this barbiturate caused only slight transient sedation (Goldberg 1954). By the next day the rabbit had recovered and seemed alert, but the day following it lapsed into a weak paretic state, described more fully below.

TABLE 5. COMPARISON OF HAEMATOLOGICAL RESULTS AND TOTAL PORPHYRIN EXCRETIONS ADDITIONAL TO NORMAL, OF 5 RABBITS TREATED WITH ALLYL-*ISO*-PROPYL-ACETAMIDE (NOS. 1, 4, 5 AND 7) AND SEDORMID (NO. 10)

rabbit no.	duration of drug (days)		Hb. (g %)	R.B.C. (10^6/mm^3)	reticulocytes (%)	total uroporphyrin loss (mg)	total coproporphyrin loss additional to normal (mg)	faecal coproporphyrin loss additional to normal (mg)
1	14	before	10·64	4·10	2·5	21·24	4·05	—
		after	11·77	4·25	1			
4	13	before	13·61	4·90	1	122·5	3·66	—
		after	14·28	4·77	1			
5	23	before	13·31	5·77	2·5	139	7·87	28·65
		after	13·77	5·05	1			
7	12	before	10·88	4·55	1	92·2	2·58	—
		after	11·42	4·71	1			
10	9	before	13·2	5·29	0·75	10·71	1·36	—
		after	13·2	5·19	1			

Mode of death

Histopathology of tissues

In rabbits 1, 3, 10 and 13, post-mortem examination of tissues was normal apart from hepatic necrosis. Rabbit 2 at post-mortem had mid-zone degenerative changes, such as vacuolation and haemorrhage, in the liver. These five rabbits had become obviously weaker in the 2 days before death, and in rabbit 10 this was accompanied by a fall in systolic blood pressure. Rabbits 2 and 3 were found dead in their cages, but rabbits 1, 10 and 13 showed a similar mode of exitus. They all had tremor of limbs and violent convulsions, and in rabbit 13 there was marked opisthotonus lasting 15 to 30 min before death.

Rabbits 7 and 12 became weak and dyspnoeic and developed a purulent nasal discharge the day before death. At post-mortem examination, bronchopneumonia was noted and confirmed microscopically. Rabbit 8 was apparently normal until the day of death, when it became weak, with drooping head, and with a mucoid discharge from eyes and nose. This rabbit died during intubation. At post-mortem the liver and kidneys were pale, and histological examination revealed vacuolation of the peripheral cells of the liver lobules and of the convoluted tubular epithelium of the kidneys. The lungs showed a patchy bronchitis with foci of collapse and pulmonary oedema. The clinical diagnosis of rabbit 'snuffles' (*Pasteurella septica*) was not confirmed bacteriologically.

Rabbit 4, which had been given an injection of barbiturate (see above), became very weak with paresis of neck and limb muscles 2 days following this injection, after having recovered from its hypnotic effect. The urinary volume had decreased,

but the urine contained no protein and merely an occasional erythrocyte on microscopic examination. There was absence of pain sensation in the right hind leg, but electromyography of the limb muscles failed to reveal any abnormality. The rabbit's condition deteriorated and 2 days after the onset of this state it was sacrificed, bled and immediately perfused with 10 % formol saline to allow investigation of the nervous system. The blood plasma was markedly opalescent. Table 6 shows the results of blood and plasma examinations, for which we are indebted to Dr F. V. Flynn, Department of Clinical Pathology, University College Hospital. A lower nephron nephrosis (with consequent uraemia) was indicated by the microscopic finding of tubular distension with flattened epithelium and fatty vacuolation of some of the convoluted tubules of the kidneys. The liver showed minor periportal fatty changes without evidence of necrosis.

TABLE 6. BLOOD ANALYSES OF RABBIT 4 (A.I.A. + BARBITURATE) COMPARED WITH NORMAL LEVELS

	rabbit 4	normal rabbit
plasma cholestrol (mg %)	410	60–100 (34)
blood urea (mg %)	308	45 (4)
plasma potassium (m.eq/l.)	6·34	4·5–5·5 (20)
plasma sodium (m.eq/l.)	140	140 (20)

Of the remaining five rabbits, nos. 6, 11 and 15 died within half an hour after intubation, and post-mortem examination failed to show any histological abnormality. Rabbit 9 became weak, with deep, laboured respiration on the day before its death. Histology of its tissues was normal. Rabbit 14 did not recover from the hypnotic effect of its dose of sedormid. Again, post-mortem histological examination was normal.

SPLENECTOMY

In rabbit 12, a period of 3 weeks rest was allowed after a course of sedormid lasting 11 days. A splenectomy was then performed, and the following day the rabbit was started on a second course of sedormid with the same dose as in the initial course. Porphobilinogen and uroporphyrin were noted, in addition to increased coproporphyrin, on the 2nd day of drug administration and rose gradually until a daily level of 9·5 and 2·2 mg of porphobilinogen and uroporphyrin, respectively, were obtained after 1 week. The rabbit then died and was found at autopsy to have bronchopneumonia.

EFFECT OF RETICULO-ENDOTHELIAL BLOCKADE

Table 7 summarizes the results of sedormid intoxication in the rabbits (nos. 32, 33 and 34) which had a simultaneous reticulo-endothelial blockade by thorium dioxide. In two of these rabbits a splenectomy had been performed before the blockade, in order to increase the concentration of thorium dioxide in the liver.

Rabbit 32 showed no change in blood haemoglobin concentration, erythrocyte, reticulocyte and platelet counts, but did develop a leucocytosis, from 3000/mm^3 to 13 400/mm^3, and a diminished clot retraction at the end of the experiment.

This rabbit also developed an abnormal urinary amino-acid pattern, from a solitary glycine spot before the experiment to an excretion of glutamic acid, glycine, taurine, glutamine and histidine before death. At post-mortem examination there was marked hepatic necrosis. Rabbit 33 survived the full course of the experiment, but 20 days later, when the same dose of sedormid was repeated, the rabbit went into profound coma from which it did not recover. The liver parenchyma at post-mortem examination appeared healthy. Rabbit 34 was found dead in its cage; there had been some infection of the splenectomy wound. Microscopic examination of the livers of rabbits 32 and 33 showed the presence of thorium dioxide mainly in the Kupffer cells, but also in small amounts throughout the hepatic cells.

A further experiment was carried out on a normal rabbit in which an external bile fistula had been performed. Administration of thorium dioxide in similar dosage to the above experiment did not cause any fall in the levels of bile porphyrins and urinary porphyrins.

Experiments on fowls

A.I.A. in gelatin capsules was administered to two Rhode Island Red chickens, each weighing 2 kg. No. 1 received the drug for 18 days and no. 2 for 17 days. Chicken 2 was also given injections of sodium allyl-(1-methylbutyl)-barbiturate (seconal) in a mean daily dosage of 37 mg during the final 7 days of its drug course.

Porphyrin and porphobilinogen excretion. In both animals there was an immediate rise in the coproporphyrin and protoporphyrin content of the excreta. Porphobilinogen and uroporphyrin were noted on the 4th and 7th day respectively (see table 8). Chicken 1 excreted greater amounts of porphobilinogen and uroporphyrin than chicken 2. The porphobilinogen was identified by means of paper chromatography (Westall 1952) as being identical with that isolated from the urine of a case of acute porphyria. Among the porphyrins contained in the excreta, uroporphyrin III and coproporphyrin III were identified by means of paper chromatography (see Methods).

Clinical and pathological results. Both chickens were active throughout the course of this experiment, although they lost an average of 30 g weight daily. The weight of their excreta progressively diminished. Their Hb and R.B.C. levels did not alter throughout the experiment. Towards the end of the experiment, both chickens became weak and lethargic, although frank paralysis was not seen. They were sacrificed by means of nembutal intravenously, bled and infused through the aorta with 10% formol saline. The examination of their nervous systems is reported by Dr J. C. B. Fenton. Histological examination of the kidneys of both these fowls showed subnuclear vacuolation in the convoluted tubules. The liver of chicken no. 1 was normal and contained porphobilinogen, but that of no. 2 showed cellular necrosis and contained no porphobilinogen.

Experiments on rats

Nine Wistar strain albino rats (175 to 204 g) were placed in metabolism cages, allowing the separate collection of urine and faeces. After a preliminary base-line period of 3 days, they were given A.I.A. by means of gastric intubation through

TABLE 7. EFFECTS OF RETICULO-ENDOTHELIAL BLOCKADE IN EXPERIMENTAL PORPHYRIA

rabbit no.	total dose thorium dioxide (ml/kg)	dose sedormid (mg/kg/day)	duration of sedormid course (days)	urinary porphobilinogen (mg/day) mean	urinary porphobilinogen (mg/day) max.	urinary uroporphyrin (mg/day) mean	urinary uroporphyrin (mg/day) max.	urinary coproporphyrin additional to normal (μg/day)	faecal coproporphyrin additional to normal (μg/day)
32	18·1	232	13	4·05	7·05	1·58	3·23	121	571
33*	13·4	218	13	1·68	3	0·69	1·63	134	—
34*	10·5	238	10	2·12	2·46	0·04	0·06	100	—

* These rabbits had their spleen removed before thorium dioxide was given.

TABLE 8. PORPHYRINS AND PORPHOBILINOGEN IN TISSUES AND EXCRETA OF 2 FOWLS TREATED WITH A.I.A.

The values in liver, bile and marrow are expressed as μg/g or μg/ml. Those in excreta refer to the mean daily output (μg/day). The coproporphyrin and protoporphyrin figures in the excreta are those additional to mean normal daily levels. The bile and bone marrow contained no porphobilinogen or uroporphyrin.

	liver				bile		marrow		excreta			
chicken	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	copro-porphyrin	proto-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin
1	10·60	32·43	6	6·32	—	—	0·25	1·34	788	757	104	1,610
2	1	44	0	1·13	451	366	—	—	761	1,165	16	791

TABLE 9. MEAN DAILY URINARY PORPHOBILINOGEN AND COPROPORPHYRIN EXCRETION OF RATS TREATED WITH A.I.A. THE COPROPORPHYRIN IS THAT ADDITIONAL TO THE MEAN NORMAL LEVEL IN EACH RAT

rat no.	A.I.A. dose (mg/kg/day)	duration (days)	porphobilinogen (mg/day)	coproporphyrin (μg/day)
1	455	18	2·64	37
2	405	22	5·72	57
3	258	13	0·39	33
4	286	7	0·042	18
5	490	15	1·27	52
6	500	13	1·27	54
7	470	8	0	30
8	490	15	2·945	36
9	350	6	0	30

TABLE 10. PORPHYRINS AND PORPHOBILINOGEN (μg/g) IN TISSUES OF RATS TREATED WITH A.I.A.

	liver				kidney				spleen			
rat no.	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin
2	0·1	0·3	316	5·3	1·42	1·13	59	15	0·32	1·15	0	2·48
5	2·38	1·2	28·4	4·4	2·23	0·79	33	12·4	0·13	0·85	0	0
6	0·85	0·55	7	0·87	0·79	0·06	0	2·67	0·1	0	0	0
7	0·27	0·43	0	0	0·23	0·15	0	0	—	—	0	—
8	0·58	0·40	145	0·91	0·77	0·91	49	0·62	—	—	0	—
9	0·26	0·30	0	0	0·48	0·31	0	0	—	—	0	—
control	0·02	0·16	0	0	0·08	0·07	0	0	0·05	0·29	0	0

a blunted metal needle (size: internal diameter 18 s.w.g. (0·048 in.), external diameter 15 s.w.g. (0·072 in.)) attached to a glass syringe. The A.I.A. powder had previously been thoroughly ground up in a mortar and suspended in 'cellophas' (I.C.I.) 4% (1 part) and water (4 parts). A useful concentration of the drug was 100 mg/ml of suspension. It was found that the appropriate dose in these rats was about 100 mg/day (400 to 500 mg/kg).

Porphyrin and porphobilinogen excretion (see table 9). The nine rats showed an immediate rise in urinary coproporphyrin. In rat 6 faecal porphyrins were also determined. The increase in ether-soluble porphyrins in the faeces greatly exceeded that in the urine, e.g. rat 6 had a mean daily increase of urinary coproporphyrin of 54μg, the rises in daily stool coproporphyrin and protoporphyrin being 115 and 436μg respectively. Seven rats excreted porphobilinogen on the 2nd (rats 1, 2, 3 and 4), 4th (rat 5), 6th (rat 6) or 11th (rat 8) day of drug administration. The mean level of porphobilinogen excretion was about 2 mg/day, but rat 2 had persistently high levels reaching a maximum of 15·2 mg/day. Freshly voided urine, containing porphobilinogen, was normally coloured and had no porphyrin fluorescence. Spectrophotometric examination on this fresh urine showed no evidence of any preformed uroporphyrin. It darkened on standing and became fluorescent, although these changes were not so marked as in the rabbit urines. Small quantities of porphobilinogen were found in the rat's stools.

Two rats (nos 7 and 9) excreted no porphobilinogen, although there was a rise in urinary coproporphyrin. Throughout the experiment they became progressively weaker, and by the 6th and 8th day, respectively, were on the point of death, when they were sacrificed (see below).

The porphyrin excreted in the urine was defined as coproporphyrin series III by means of paper chromatography (see Methods). Porphobilinogen was isolated from the urine as characteristic crystals using the method of Cookson & Rimington (1954).

Clinical and pathological results. All rats showed some loss of weight (mean loss 2·5 g/day) and diminished appetite, although not as severely as did rabbits. They did not have the profound constipation noted in most rabbits, although in some rats there was a diminution in stool weight from a normal mean 5 g/day to a mean of 3·1 g/day. They appeared to be more dazed than the rabbits for several hours after A.I.A. administration. There was no clinical evidence of paralysis. During the course of the experiment, rats 1 and 2 were each exposed to a carbon arc lamp at 1 ft. distance on two separate occasions, lasting 15 and 30 min respectively. Neither rat showed any sign of photosensitivity. The clinical course of rats 7 and 9 has been described. The other seven rats remained mobile and alert. They were sacrificed at the end of the experiment. Chemical analysis of tissues is summarized in table 10. Those rats excreting porphobilinogen in the urine had porphobilinogen in the liver, while rats 7 and 9 had porphobilinogen neither in their urines nor livers.

Histology of the livers in those rats excreting porphobilinogen was normal. The liver of rat 7 showed fatty degeneration, but that of no. 9 was histologically normal. Amino-acid chromatograms of the urines, before and at the end of the experiment,

were done in rats 1, 2, 3, 4, 5, 7 and 10. No change in the amino-acid pattern was noted. Histological examination of the nervous system of rats 1, 2, 3 and 4 is reported by Dr J. C. B. Fenton.

Discussion

A question, important to our understanding of human acute porphyria, is whether or not the known porphyrins or their precursors, or unknown substances, released by the pigment dyscrasia, cause the clinical symptoms of this disease. Recent work on the pharmacological action of carefully purified porphyrins and porphobilinogen (Goldberg, Paton & Thompson 1954) afforded no evidence for any direct action of these substances, and the finding that porphobilinogen is probably a normal precursor of porphyrins and haem (Falk, Dresel & Rimington 1953) renders less likely the former assumption that the symptomatic disturbances in acute porphyria were due to porphobilinogen. The work of Schmid & Schwartz (1952) suggested a direct association between the chemical disturbance induced by sedormid and the gastro-intestinal and neurological symptoms which they observed in rabbits. In our observations, however, the hypnotic action of sedormid obscured the clinical features of the rabbit porphyria. Allyl-*iso*propyl-acetamide allows an experimental porphyria without hypnosis. The separation of these two properties, excess porphyrin and porphobilinogen production and hypnosis, is in itself noteworthy, since some of the most potent drugs disturbing porphyrin metabolism, e.g. sulphonal, trional, sedormid and certain barbiturates, are hypnotics.

Using either sedormid or A.I.A., we have confirmed the association of the chemical and gastro-intestinal features of experimental porphyria, but have failed to observe any paralyses, with one exception (rabbit 4) where a barbiturate was given in addition to A.I.A. The clinical and histo-pathological pictures were here complicated by uraemia (see report by Dr Fenton). In order to study this point more fully, experiments were carried out on fowls, animals particularly susceptible to metabolic derangement of the nervous system. Fowl 2 was given a barbiturate in addition to A.I.A. In these animals there was no evidence, clinical or histopathological, of any selective damage to nervous tissues. The experiments in which rats were intoxicated with A.I.A. showed that, in spite of high levels of porphobilinogen and porphyrin excretion in the urine, the animals remained normally mobile and alert, and histopathological examination of the nervous system revealed no lesion. Another point of dissimilarity between rabbit experimental porphyria and human acute porphyria is the absence of hypertension, although this is a common finding in acute porphyria.

The haematological data agree with the normal blood levels obtained in active phases of human acute porphyria. It must be emphasized that these figures are concentration levels, and blood volumes, which might alter in the progressive wasting states of these rabbits, were not measured. Courtice & Gunton (1949) have noted an increase in the plasma volume of rabbits in which cabbage was added to the normal diet. On the other hand, Henschel, Mickelson, Taylor & Keys (1947) have shown that the absolute plasma volume *increased* slightly in a group of thirty-two men undergoing a period of semi-starvation in which about one-quarter of the

body weight was lost, approximately the same proportion as lost by the rabbits studied. If the latter findings are applicable to our experiments, they would suggest that the undiminished haemoglobin concentration levels in the rabbits, implied, at least, maintenance of the total amount of haemoglobin throughout a period during which large quantities of porphyrins were excreted. Table 11 attempts to estimate the ratio of daily porphyrin loss to normal daily protoporphyrin formation in three of these rabbits (nos. 4, 5 and 7). This ratio is about 1 to 2. It is suggested that if the porphyrin excretion represented a deviation of porphyrin from normal haem production, this would have resulted in a recognizable anaemia. Drabkin (1951) has suggested that the porphyrins excreted in the human porphyrias might be the result of an 'under-utilization' owing to diminished globin supply, rather than of an 'overproduction' of these pigments. Our findings in this rabbit porphyria would point to an 'overproduction' rather than an 'under-utilization'. Another factor strengthening this view is the diminished urinary glycine excretion noted by paper chromatography in those rabbits (nos. 4, 5, 6, 7 and 12) with the high total porphyrin output of 126, 147, 38, 95 and 88 mg, respectively, during the periods under consideration.

TABLE 11. ESTIMATED RATIO (EXPRESSED AS A PERCENTAGE) OF DAILY PORPHYRIN EXCRETION TO NORMAL DAILY PROTOPORPHYRIN FORMATION IN 3 RABBITS TREATED WITH A.I.A.

The normal daily protoporphyrin formed was calculated from the following data: (*a*) blood volume in the normal rabbit is 70 ml/kg (6) and (*b*) average life span of the rabbit erythrocyte is about 68 days (22). (*c*) haemoglobin level as determined at commencement of experiment (see table 5).

rabbit no.	body weight (kg)	probable blood volume	estimated normal proto-porphyrin formed (mg/day)	determined porphyrin loss (mg/day)	$\frac{\text{porphyrin loss}}{\text{normal porphyrin formation}}$ (%)
4	4·12	288	19·6	9·8	50·0
5	3·69	258	17·7	7·65	43·3
7	3·14	220	15·0	7·9	52·7

Such a diminution of glycine excretion was not observed in other rabbits treated with the same drugs, nor in rabbits treated with certain barbiturates (Goldberg 1954) where the total porphyrin excretion was much less. This suggests that in the high porphyrin excretors there was some encroachment on the glycine pool; but there is, however, still the possibility (in rabbit 4) that the diminished glycine excretion is due to renal failure, for it is known that in the human, amino-acid excretion in the urine decreases in renal failure (Dent 1954). Glycine is known to be a precursor of porphyrin (Shemin & Rittenberg 1946).

Table 12 groups together and compares six 'good porphyrin excretors' (above 6 mg uroporphyrin/day) and four 'poor porphyrin excretors' (below 2 mg uroporphyrin/day). These ten animals, out of a series of fifteen treated with A.I.A. or sedormid, clearly fell into two groups. The comparison of these groups emphasizes

the role of the liver in the production of porphobilinogen. All poor excretors had pathological evidence of severe hepatic damage and porphobilinogen was not present in the livers. The pronounced amino-aciduria might be an expression of this hepatic damage. On the other hand, the livers of good excretors showed either normal parenchymatous tissue or minor changes, with porphobilinogen present in four out of five examined. The significance of the diminished urinary glycine spot in these good excretors has been discussed. The importance of the liver in porphyrin metabolism has been emphasized previously (Prunty 1945; Watson *et al.* 1945). The marked fall in the urinary excretion of porphobilinogen and porphyrin 1 to 2 days before death of the 'poor excretors' suggests a failure of synthetic activity in the liver due to progressive intoxication. These findings also suggest that the difference between good and poor excretors lies in the inherent ability of the liver of an individual rabbit to deal effectively with the drug (A.I.A. or sedormid). One might speculate upon the possibility of this difference being genetically determined, and draw a comparison with human acute porphyria in which a genetically determined aberration of metabolism (probably affecting the liver) is known to exist.

TABLE 12. COMPARISON OF 'GOOD' AND 'POOR' EXCRETORS

rabbit no.	mean uroporphyrin excretion (mg/day)	final urinary amino-acids	liver	
			porphobilinogen	histology
4	10·21	glycine diminished	+ + +	minor periportal fatty change
5	6·32	glycine diminished	animal still on drug after 23 days	
6	11·62	glycine diminished	+ + +	some fatty change, no necrosis
7	10·26	glycine diminished	0*	parenchyma normal
11	6·6	not determined	+ + +	parenchyma normal
12	11·29	glycine diminished	+ + +	parenchyma normal
1	1·52	many present	0	cellular necrosis
2	0·43	not determined	0	mid-zone degenerative changes
3	1·47	many present	0	cellular necrosis
10	1·53	many present	0	cellular necrosis

* No drug given during last 2 days of life. Died from bronchopneumonia.

Of the remaining five rabbits, three could not be classed as 'good' or 'poor' excretors owing to lack of adequate data (nos. 9, 14 and 15); one (no. 8) died from an infection before maximum porphyrin levels were reached, and the remaining rabbit (no. 13) would have been classified as a good excretor (8·8 mg uroporphyrin/day) had it not died with hepatic necrosis 6 days after the cessation of sedormid administration.

In attempting a comparison between human acute porphyria and experimental porphyria in the rabbit, emphasis has so far been laid on the difference between

these two states. There are, however, certain similarities, viz. the chemical similarity of the porphyrins and porphobilinogen excreted, the relatively undisturbed blood picture and the constipation, the gaseous distension seen radiologically (Mason, Courville & Ziskind 1933), although it cannot be excluded that the gastro-intestinal symptoms in the rabbit might be a direct result of the drug employed. The histological changes in the liver, where any such have occurred, are more severe than those usually described for human acute porphyria. On the other hand, several rabbit livers were histologically normal, just as in many cases of human acute porphyria. Certain drugs containing allyl groups are known to be hepatoxic (Popper 1936; Goldberg 1954), and this must be considered in relation to the pathological changes seen in some of our rabbits. The histological changes in the renal tubules of rabbits 4 and 8 are reminiscent of the lower nephron nephrosis described by Prunty (1946) in a human case of acute porphyria. Bronchopneumonia, common as a final cause of death in acute porphyria, occurred in two rabbits.

Table 13 presents the chemical structures of the drugs used and their effects on the porphyrin metabolism of rabbits. From this summary it may be concluded that at least one allyl group, together with an acid amide, ureide or a cyclic ureide, as in the barbiturate series, is the constant chemical structure in the compounds found to be effective.

The results of the experiments using reticulo-endothelial blockade by means of thorium dioxide agree with the work of Vannotti (1937), who found that a preliminary reticulo-endothelial blockade did not diminish porphyrin excretion in normal rabbits, but decreased porphyrin formation in lead-intoxicated rabbits. The three rabbits in the present experiment behaved as 'poor excretors'. This might be due to the effect of thorium dioxide on the Kupffer cells or the hepatic cells. It is possible, however, that all three of these rabbits would have been 'poor excretors' even without thorium dioxide.

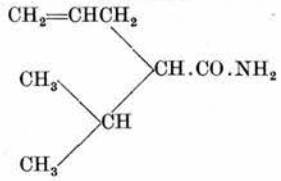
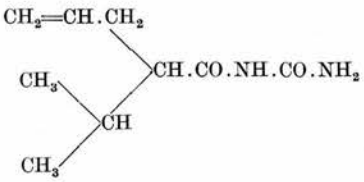
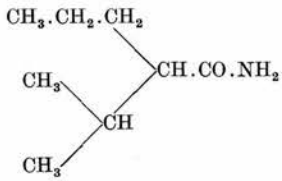
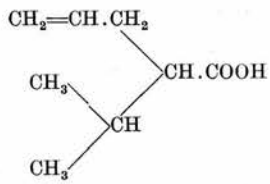
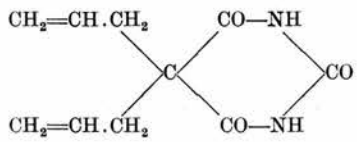
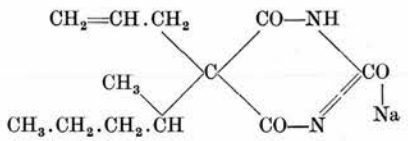
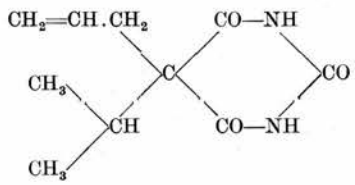
The results of splenectomy in rabbit 12 indicate that porphobilinogen, uroporphyrin and coproporphyrin can be formed in the absence of the spleen.

The porphyria induced by A.I.A. in rats showed a certain similarity to that in the rabbits, although points of difference were also noted. The distinction between 'good excretors' and 'poor excretors' was more clearly evident in rats than in rabbits. The rats tolerated a relatively higher dose of A.I.A., were much less constipated than the rabbits and showed no change in their urinary amino-acid patterns. Both the rat and rabbit illustrate that, as in the normal animal, the main route of excretion of the ether-soluble porphyrins is in the faeces. The main route of excretion of porphobilinogen appears to be the urine, although small amounts are excreted in the stool.

All the drugs used in this investigation (with the exception of the barbiturates) were most generously provided by the Hofmann La Roche Co., Basle, to whom we express our gratitude.

We wish to thank Dr R. A. B. Drury for histopathological examination of animal tissues and Dr F. V. Flynn for several biochemical determinations. Dr C. E. Dent advised on amino-acid investigations and also helped with valuable criticism. We

TABLE 13. CHEMICAL STRUCTURE AND EFFECT OF CERTAIN DRUGS ON THE PORPHYRIN METABOLISM OF RABBITS

drug	structure	effect on urinary porphyrin excretion
allyl-*iso*propyl-acetamide (A.I.A.)		+ + +
allyl-*iso*propyl-acetyl-urea (sedormid)		+ + +
n-propyl-*iso*propyl-acetamide		− − −
allyl-*iso*propyl-acetic acid		±
diallyl-barbituric acid (dial)		+ +
sodium allyl-(1-methyl butyl)-barbiturate (seconal)		+
allyl-*iso*propyl-barbituric acid		+

+ + + Very marked effect (much coproporphyrin, porphobilinogen and uroporphyrin).
+ + Marked effect (much coproporphyrin, some porphobilinogen and uroporphyrin in three out of eight rabbits).
+ Moderate effect (coproporphyrin only).
± Slight coproporphyrin rise only.
− − − No effect.

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Notes on histopathological studies of the nervous system in experimental porphyria

By J. C. B. Fenton

No evidence of unequivocal myelin degeneration has been found in rabbits, fowls and rats intoxicated with allyl-*iso*propyl-acetamide. In one rabbit, myelin changes were observed, but these were considered to be atypical of true myelin degeneration and could be attributed to causes other than the direct action of the drug.

In view of the importance of the neurological symptoms in acute porphyria and the finding by Schmid & Schwartz (1952) of paralysis in sedormid-intoxicated rabbits, a histopathological study has been made on nervous tissue taken from two rabbits, two fowls and four rats, intoxicated with A.I.A.

Methods

Rabbits and fowls were sacrificed by means of intravenous nembutal and then immediately perfused through the aorta with 10% formol saline in order to avoid artifacts, caused by handling of unfixed tissues. In the case of rats, the abdominal and thoracic organs, fur and skin were removed, and the residual parts of the head and body immersed directly in 10% formol saline. Portions of the peripheral and central nervous systems were removed and stained by Swank & Davenport's (1935) modification of the Marchi method.

Results

Rabbit 4. This animal was the only one in which there was any clinical suspicion of paralysis. A barbiturate had been given in addition to A.I.A. On examination of the brachial, sciatic and vagus nerves, extensive blackening of many fibres was found (see figure 5, plate 11). There were numerous fine black droplets scattered evenly throughout the white matter of the spinal cord, but the midbrain and cerebrum appeared normal. This at first suggested myelin degeneration, but closer examination revealed that the myelin sheaths were regular in outline and no globules of free lipoid could be seen. A similar type of Marchi reaction has been reported by Swank & Bessey (1950) under conditions of starvation. Rabbit 4 suffered from a progressively severe anorexia, presumably associated with the drug administration and also with the terminal uraemia. The normal electromyogram in this rabbit gives additional support to the suggestion that the extensive myelin changes had been produced by starvation.

Rabbit 6. No myelin degeneration was found in a sciatic nerve from this rabbit.

Chickens 1 *and* 2. In the final 24 h of intoxication, both of these animals became very weak, making clinical assessment of paralysis difficult. Examination of the peripheral and central nervous systems of both animals failed to reveal any myelin degeneration.

Rats 1, 2, 3 *and* 4. None of these animals had shown any clinical suspicion of paralysis. No evidence of myelin degeneration was found in their peripheral or central nervous systems.

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Fate of Porphobilinogen, Administered Enterally or Parenterally, in the Rat

By A. GOLDBERG
Nuffield Unit for the Investigation of Pyrrole Pigment Metabolism, Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

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Porphobilinogen is excreted in the urine in large quantities in acute porphyria and is always found in the livers of fatal cases of this disease. In experimental porphyria in animals induced by sedormid (allyl*iso*propylacetylurea) (Schmid & Schwartz, 1952) or allyl*iso*propylacetamide (Goldberg, 1953), porphobilinogen is likewise found in the urine and liver. Previous reports on the behaviour of this substance when administered to experimental animals are conflicting, probably because the porphobilinogen used was only partially purified or was available in amounts too small for adequate investigation. Thus Waldenström & Wendt (1939) injected partially purified porphobilinogen into rabbits and found it to be excreted in the urine, while Prunty (1945) failed to find any in the urine under similar conditions. Goldberg, Paton & Thompson (1954) injected porphobilinogen, which had been isolated by Westall (1952), intravenously into one rabbit with an external bile fistula and found traces of uroporphyrin in the urine and a slight increase in the excretion of bile porphyrin.

The following experiments with porphobilinogen were done in order to clarify its mode of excretion in an experimental animal. It is suggested that the results of these studies in the rat may be relevant to human acute porphyria. It was found that porphobilinogen, when administered parenterally to the rat, is excreted into the urine mainly by glomerular filtration. If it is given enterally or parenterally, it is not found in the livers of rats subsequently killed, at a time when the plasma still contains porphobilinogen. These findings suggest that the porphobilinogen, found in the livers of rats with experimental porphyria, has been formed there and has not been transported to the liver from an extrahepatic site.

Falk, Dresel & Rimington (1953) showed that porphobilinogen behaved as a precursor of certain porphyrins in a haemolysed chicken-erythrocyte

system. In the present work, a small but significant conversion of porphobilinogen into coproporphyrin III and uroporphyrin III has been detected in the rat. A brief preliminary communication concerning this work has appeared (Goldberg & Rimington, 1954*a*).

METHODS

General

The Wistar-strain rats used weighed between 150 and 214 g. with the exception of rat no. 7 which weighed 112 g. Rats nos. 9 and 13 were female. They were placed in metabolism cages. In studies lasting 2 weeks, separate daily urinary and faecal specimens were analysed every second day for porphyrins, the specimens not determined on the day of excretion being stored at 2°. In experiments where urine was collected at frequent intervals, e.g. half-hourly or hourly, micturition was induced by a 'tail-pulling' procedure similar to that described by Hutschenreuter (1933). In collecting urine from metabolism cages, the cage was washed with water, until the washings were free from porphobilinogen (see below). In the experiment on rat no. 9, where hourly urine specimens were taken for 20 hr., the interior of the metabolism cage was washed 4 times at the end of each hr. with a total vol. (each hr.) of 200 ml. water.

The porphobilinogen used had been isolated in the crystalline form (Westall, 1952; Cookson & Rimington, 1954) and was administered as the hydrochloride in 0·9% (w/v) NaCl, using 0·5 ml. for parenteral injection and 1 ml. for enteral administration. Intravenous injections were given into a tail vein, subcutaneous into the dorsal area of the rat. All enteral administration was carried out by gastric intubation as described by Goldberg & Rimington (1954*b*).

Haematological values were determined as described (Goldberg, 1954).

Isolation and determination of porphyrins

Urine. Coproporphyrin was extracted by the method of Schwartz, Zieve & Watson (1951). Uroporphyrin from the same specimen of urine was obtained by combining the aqueous and sodium acetate washings from the coproporphyrin extraction, and bringing these to pH 3·0–3·2. The washings were then shaken with ethyl acetate, the aqueous layer was discarded and uroporphyrin extracted with 0·6 N-HCl.

Faeces. Coproporphyrin and protoporphyrin were extracted by the method of Schwartz & Wikoff (1952) with the following modifications. The ethyl acetate was shaken with 20–30 ml. 0·005% aqueous I_2 immediately after extraction of porphyrins with 3 N-HCl. The aqueous layer was discarded and a further extraction with 3 N-HCl carried out, in order to obtain any porphyrin precursor. This was not found in any specimen. The final coproporphyrin extraction in 0·1 N-HCl was not shaken with $CHCl_3$. Uroporphyrin was obtained from the sodium acetate washings of the ethyl acetate layer during the above procedure. These acetate washings were dealt with in the same way as described for urinary uroporphyrin.

The porphyrins, so extracted from urine and faeces, were determined by the method of Rimington & Sveinsson (1950) using a Beckman spectrophotometer, model D.U.

Paper chromatography of porphyrins. This was carried out as described (Goldberg, 1954).

Isolation and determination of porphobilinogen

Urine and tissues. Porphobilinogen was determined as described (Goldberg, 1954). It was assumed that 1 mg. of porphobilinogen is equivalent to 130 Vahlquist (1939) units (Westall, 1952).

Faeces. To a weighed amount of faeces in a centrifuge tube, sufficient water was added to give a thin paste. Clarification was effected by adding 10% (w/v) lead acetate in 3% (w/v) acetic acid, in a volume one-tenth of that of the diluted faeces, mixing and then centrifuging. The supernatant was decanted and excess Pb^{2+} was precipitated with a solution containing NaH_2PO_4 (123 g./l.) and K_2HPO_4 (138 g./l.). The mixture was centrifuged and the supernatant analysed for porphobilinogen as described for urine.

Plasma. Protein was precipitated by adding 0·33 vol. of 20% (w/v) trichloroacetic acid to centrifuged plasma as obtained *post mortem*. The mixture was then centrifuged and the supernatant tested for porphobilinogen. In the living rat 0·1 ml. of blood from the tail was sucked into a 0·1 ml. pipette, previously rinsed with heparin. The blood was discharged into 1·5 ml. of 5% (w/v) trichloroacetic acid contained in a centrifuge tube. This was thoroughly shaken, centrifuged and the supernatant tested for porphobilinogen. The observation that porphobilinogen does not enter erythrocytes (Goldberg & Rimington, 1954*b*) was confirmed in the present study. The blood haematocrit for the rat was taken to be 40 (Wintrobe, 1951) and plasma porphobilinogen was calculated on this basis.

Paper chromatography. This was done as described by Westall (1952) and Cookson & Rimington (1953).

RESULTS

Intravenous injection of porphobilinogen

Excretion pattern of porphobilinogen and porphyrins. Rats nos. 1 and 2 were given intravenous injections of 9·04 and 9·4 mg., respectively, of porphobilinogen after a preliminary period of 8 and 7 days, respectively, during which daily faecal and urinary porphyrins were determined. These determinations were continued during the succeeding 8 days. Porphobilinogen was rapidly excreted in the urine of both rats. It appeared in the urine within 10 min. of injection and was detectable until 8 hr. later. In rat no. 1 specimens of urine were collected every 2 hr. during the 12 hr. period after injection. The results of porphobilinogen, uroporphyrin and coproporphyrin determinations on these specimens are illustrated in Fig. 1. This shows that nearly half of the total porphobilinogen excreted in the urine (6·07 mg.) was passed in the first 2 hr. after injection, while the excretion of the small quantities of uroporphyrin (4·59 μg.) and coproporphyrin (6·85 μg.) was more protracted. A smaller yield of porphobilinogen was obtained in the urine of rat no. 2, viz. 2·85 mg., of which 2·6 mg. were excreted in the first 3 hr. after injection. This rat excreted a total of 4·95 μg. of uroporphyrin and 6·0 μg. of coproporphyrin in the 24 hr. following injection.

Paper chromatography of these porphyrins showed that they behaved as uroporphyrin III and coproporphyrin III, respectively. The porphobilinogen excreted in the urine was indistinguishable from crystalline porphobilinogen by paper chromatography, using butanol–acetic acid (Westall, 1952). Furthermore, the lactam derivatives (Cookson & Rimington, 1953) of the porphobilinogen excreted in the urine and of crystalline porphobilinogen, behaved identically on a paper chromatogram.

Table 1 summarizes the mean daily faecal coproporphyrin and protoporphyrin values before and after administration of porphobilinogen in rats nos. 1 and 2. These figures are similar in each rat. Paper chromatography of these porphyrins both before and after porphobilinogen administration demonstrated that some dicarboxylic porphyrin (similar in properties to protoporphyrin) as well as coproporphyrin had been extracted by 0·1 N-HCl and thus the coproporphyrin determinations in Table 1 are too high. However, an amount of dicarboxylic porphyrin similar to that of coproporphyrin (as judged by fluorescence on paper chromatograms) was extracted by 0·1 N-HCl in each case. It was therefore considered that no significant change had occurred in faecal porphyrins as a result of porphobilinogen administration.

Neither rat showed any abnormal symptoms during the 8-day period following the porphobilinogen injection. Haemoglobin contents, red cell numbers and reticulocyte counts were determined in both rats, before and at the end of the experiment and one day before and one day after the injection. No significant changes in these haematological values were observed.

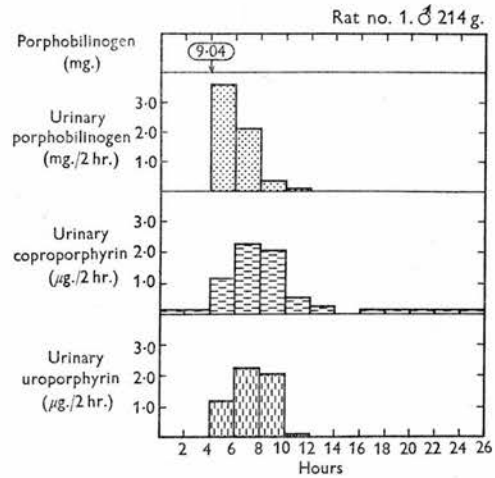

Fig. 1. Urinary excretion of porphobilinogen and porphyrins after the intravenous injection of porphobilinogen in rat no. 1.

Table 1. *Urinary and faecal porphyrin determinations made with rats nos. 1, 2 and 3, before and after porphobilinogen (Pbg.) administration, by the intravenous (i.v.) or enteral (g.i.) routes*

Urinary and faecal porphobilinogen were recovered after its administration. The durations of the 'before' periods for rats nos. 1, 2 and 3 were 8, 7 and 6 days respectively; those for 'after' were 3 days in each case.

Rat no.	Weight (g.)	Dose of Pbg. (mg.)	Mean urinary coproporphyrin (μg./day)		Mean faecal coproporphyrin (μg./day)		Mean faecal protoporphyrin (μg./day)		Pbg. recovered		
			Before	After	Before	After	Before	After	Faeces (mg.)	Urine (mg.)	% (urine + faeces)
1	266	9·04 (i.v.)	1·7 (±0·6)	3·7 (±2·2)	134 (±33)	78 (±16)	135 (±39)	130 (±31)	0·097	6·07	68
2	172	9·4 (i.v.)	1·3 (±0·4)	3·7 (±1·9)	53 (±10·7)	42 (±9·1)	98 (±25)	122 (±36)	0·055	2·85	31
3	214	3·68 (g.i.)	1·6 (±0·6)	2·1 (±1·4)	39 (±12·6)	27 (±6·7)	66 (±15·2)	79 (±29·2)	1·676	0	45

Rats killed within 30 *min.* Porphobilinogen (2·2, 2·0 and 2·14 mg.) was injected by vein into rats nos. 4–6 and these were killed after 30, 30 and 10 min., respectively. The porphobilinogen contents of the tissues of these rats are summarized in Table 2. The urine, plasma and kidney always contained porphobilinogen, while liver, spleen, brain, bone marrow and Harderian glands gave consistently negative results. Subcutaneous fat and lungs contained porphobilinogen, while skeletal and cardiac muscles (thoroughly washed free of blood) of rat no. 6 contained porphobilinogen. The plasma porphobilinogen level of rat no. 6 was approximately 4 times that of rats nos. 4 and 5 (killed at 30 min.). Small amounts of porphobilinogen were found in the gut contents.

Subcutaneous injection of porphobilinogen

Rats killed within 30 *min. after single injection.* Porphobilinogen (2·0 and 2·62 mg.) was injected into rats nos. 7 and 8, respectively, and the animals were killed 30 min. after the injection. The pattern of urinary and tissue porphobilinogen (Table 2) was similar to that found after intravenous injection. The plasma porphobilinogen level was higher than that found in rats nos. 4 and 5.

Rats killed 20·25 *hr. after half-hourly injections, maintained for* 20 *hr.* In experimental porphyria in rats induced by allyl*iso*propylacetamide (Goldberg & Rimington, 1954*b*), porphobilinogen is consistently found in the liver in high concentration, also in the kidney and urine, and sometimes in the plasma. This hepatic porphobilinogen could have been formed in the liver or at an extrahepatic site, e.g. the bone marrow, and transported by the blood stream to the liver. The very rapid excretion of porphobilinogen in the urine and the absence of porphobilinogen from the liver in the above experiments, when the plasma still contained porphobilinogen, made such an extrahepatic site unlikely. However, it was still possible that the liver might take up porphobilinogen from an extrahepatic site if porphobilinogen were present in the plasma for any length of time. To determine the approximate length of the period during which significant plasma levels are maintained in experimental porphyria, rat no. 12 was given allyl*iso*propylacetamide by gastric intubation. Within 20 hr. this rat had excreted porphobilinogen in the urine. It was killed and porphobilinogen was found in the liver and kidney (Table 2).

In order to mimic the effects of continuous production of porphobilinogen by a possible extrahepatic site throughout this period, 1 mg. of porphobilinogen was injected subcutaneously into rat no. 9 every 30 min. for 20 hr. The initial two doses were given together at the beginning of the experiment. Urinary excretion of porphobilinogen

Table 2. *Tissue porphobilinogen (Pbg.) determinations of rats* (4–11), *given porphobilinogen, intravenously (i.v.) subcutaneously (s.c.) or by gastric intubation (g.i.)*

Rat no. 12 was given 50 mg. of allyl*iso*propylacetamide (A.I.A.). Duration refers to period between porphobilinogen or A.I.A. administration and killing of animals. Porphobilinogen results are expressed as μg. total porphobilinogen (urine and gut contents) or as μg./g. or μg./ml. (the remaining tissues): '+' indicates a trace.

Rat no.	Body wt. (g.)	Dose Pbg. (mg.)	Duration (min.)	Porphobilinogen: Urine (total)	Gut (total)	Kidney	Plasma	Liver	Skeletal muscle	Fat	Lungs	Heart muscle	Pbg. recovered (μg.)	Pbg. recovered (%)
4	150	2·2 (i.v.)	30	854	+	50	11·3	0	*	*	*	*	976	43
5	174	2·0 (i.v.)	30	805	2·5	42·5	8·3	0	0	11·3	9·1	*	966	48
6	160	2·14 (i.v.)	10	4	+	124	38	0	10·6	9·3	8	0	1 305	61
7	112	2·0 (s.c.)	30	645	+	75	27·6	0	*	+	+	8	1 186	59
8	151	2·62 (s.c.)	30	719	10	100	26·2	0	5	16·5	10·6	0	1 021	39
9	150	40·0 (s.c.)	1215	30 160	280	69·5	27·6	0	0	7·3	6·9	0	31 734	79
10	190	3·4 (g.i.)	450	168	2122	0	0	0	0	0	0	0	2 290	67
11	197	4·85 (g.i.)	120	87	1750	0	2·1	0	0	0	0	0	1 955	40
12	174	0† (g.i.)	1200	515	9·8	10·7	0	28·6	0	0	0	0	806	—
13	142	Normal control	—	0	0	0	0	0	0	0	0	0	—	—

* Not tested.

† 50 mg. A.I.A.

Table 3. *Porphyrin determinations (μg./g. or μg./ml.) in tissues of rats which had been given porphobilinogen*

Uroporphyrin was not found in any of the tissues of these rats.

Rat no.	Liver		Kidney		Blood	
	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin
4	0·04	0·02	0·01	0·04	0	0·06
6	0·23	0·16	0·12	0·13	0·02	0·26
7	0·09	0·11	0	0·25	0	0
9	0·02	0	0·31	0·57	0	0
13 (control)	0·02	0·16	0·08	0·07	0·02	0·49

was determined hourly and urinary uroporphyrin and coproporphyrin, as well as plasma porphobilinogen were determined every 2 hr. (Fig. 2). The rat was killed 15 min. after the final injection and tissues were analysed for porphobilinogen and porphyrins (Tables 2 and 3). The liver did not contain porphobilinogen, while the gut contents showed a significant amount of porphobilinogen, presumably from passage through the bile ducts. During the 20 hr. period of the experiment the rat showed no pathological symptoms.

Inspection of Fig. 2 shows that during the latter 10 hr. of this experiment the hourly urinary excretion levels of porphobilinogen were remarkably constant—a mean of 1530 ± 151 μg. (S.D.)/hr. The mean plasma porphobilinogen level during this period was $31 \pm 7{\cdot}4$ μg. (S.D.)/ml. Thus the renal clearance for porphobilinogen in this rat was 0·82 ml./min. or 0·55 ml./min./100 g. of body weight. This figure is in good agreement with inulin clearance found in the rat (Smith, 1951) and would therefore suggest that porphobilinogen is filtered by the glomeruli and not reabsorbed to a significant extent.

Table 3 summarizes the liver, kidney, and blood porphyrin determinations of rats nos. 4, 6, 7 and 9, which had been given porphobilinogen parenterally. There is no significant elevation of the porphyrin levels.

Enteral administration of porphobilinogen

Excretion pattern of porphobilinogen and porphyrins. A study similar to that described for rats nos. 1 and 2, was carried out in rat no. 3. After a preliminary period of 6 days, 3·68 mg. of porphobilinogen were given by gastric intubation. Daily urinary and faecal porphobilinogen and porphyrin contents were determined during the base-line period and in the 7 days following porphobilinogen administration (Table 1). There was no significant change in the porphyrin levels in the urine or faeces. No porphobilinogen appeared in the urine of this rat (cf. rats nos. 10 and 11, below), but 45% of the porphobilinogen administered was

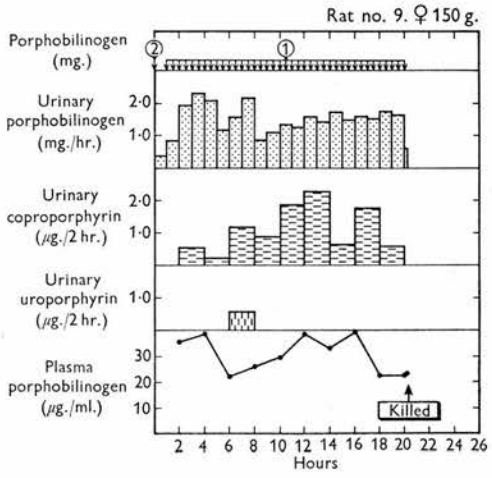

Fig. 2. Effects of repeated subcutaneous injections of porphobilinogen for 20 hr. on urinary porphobilinogen and porphyrin excretion and on plasma porphobilinogen. For experimental details see text.

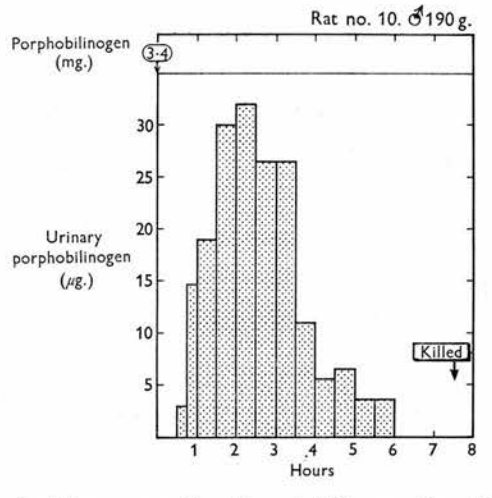

Fig. 3. Urinary excretion of porphobilinogen after intragastric intubation of 3·4 mg. of porphobilinogen.

recovered in the faeces. Traces of porphobilinogen were present in the faeces up to the sixth day after porphobilinogen administration.

Rat killed after 7·5 hr. Rat no. 10 was given 3·4 mg. of porphobilinogen by gastric intubation. Urinary porphobilinogen determinations were done every 30 min. (Fig. 3). Porphobilinogen was detected in the urine within the second half-hour and was excreted in small amounts until 6 hr. after its administration. Maximum excretion in the urine occurred between 2 and 2·5 hr. Between 6 and 7·5 hr., when the rat was killed, no porphobilinogen was detected in the urine. After death 2·12 mg. of porphobilinogen was recovered from the intestinal contents. It was notable that in spite of this large quantity of porphobilinogen in the gut, it was not excreted in the urine in the last 1·5 hr. and this would suggest that porphobilinogen is only absorbed in the upper part of the rat intestine. Porphobilinogen was not found in the other tissues (Table 2).

Rat killed after 2 hr. Rat no. 11 was given 4·85 mg. of porphobilinogen by gastric intubation and killed 2 hr. later in order to see whether porphobilinogen could be detected in the liver during a phase of maximal absorption of porphobilinogen from the gut (see above). Urinary porphobilinogen determinations, as in rat no. 10, showed the excretion of 11·3, 30 and 46 μg. of porphobilinogen in the second, third and fourth half-hour periods, respectively, after administration. The rat was then killed and 1·75 mg. of porphobilinogen were recovered from the intestinal contents. The plasma contained 2·1 μg. of porphobilinogen/ml., whilst 0·104 mg. of porphobilinogen was recovered from gut tissues after they had been thoroughly washed with normal saline. Porphobilinogen was not present in liver, kidney, muscle, fat, Harderian gland or brain (Table 2). No porphyrins were detectable in the liver.

In vitro *incubation of porphobilinogen with gastro-intestinal contents of a rat*

This experiment was carried out to investigate further the absence of conversion of porphobilinogen into porphyrins in the gut, which was demonstrated in rat no. 3. A rat was killed and its gastro-intestinal contents were mixed with sterile normal saline to give a total vol. of 23 ml. 4·5 ml. of this were pipetted into each of four 50 ml. conical flasks. Flasks nos. 1 and 2 were immersed in boiling water for 5 min. and 1 mg. of porphobilinogen in 0·56 ml. of sterile normal saline was added to flasks nos. 2 and 4. The pH of each of the contents of these flasks was about 6·6. The flasks were then plugged with cotton wool and incubated for 4 hr. with shaking at 90–100 oscillations/min. at 37°. The contents were then analysed for porphobilinogen and porphyrins (Table 4).

The results show that a 59 and 52% recovery of porphobilinogen was achieved from flasks nos. 2 and 4, but there was no significant change in the porphyrin content in any flask, with the exception of a small quantity of uroporphyrin (0·71 μg.) found in flask no. 2.

DISCUSSION

The results show that most of the porphobilinogen administered parenterally is excreted in the urine and that this excretion is rapid; small amounts, however, are recovered in the faeces. Given, however, by the enteral route, it is mainly excreted unchanged in the faeces, although small amounts, (in the case of rat no. 10, one-twentieth of the porphobilinogen given) may be excreted in the urine. The excretion behaviour of the naturally occurring porphyrins (uroporphyrin, coproporphyrin and protoporphyrin), after enteral and parenteral administration to experimental animals, has been studied previously. Fischer (1916) and Günther (1922) found that uroporphyrin given parenterally to mice and rabbits, respectively, is mainly excreted in the urine, while Fischer (1915) found that uroporphyrin, when taken by himself by mouth, was passed unchanged in the stool. On the other hand, coproporphyrin III, given orally or parenterally to rats, is not passed in the urine, but recovered in the stool (Hoffbauer, Watson & Schwartz, 1953). Protoporphyrin injected parentally into dogs with a bile-renal fistula was not accounted for in the porphyrins excreted thereafter, apart from traces of material resembling deuteroporphyrin (Watson, Pass & Schwartz, 1941). The excretion of porphobilinogen thus resembles more that of uroporphyrin than that of coproporphyrin or protoporphyrin. The mean percentage recovery of the porphobilinogen administered, parenterally or enterally, was 53 ± 14. This compares with the 50% recovery of

Table 4. *Incubation of porphobilinogen with gastro-intestinal contents of a rat*

An equal volume of a mixture of gastro-intestinal contents in sterile 0·9% (w/v) NaCl was added to each of flasks nos. 1–4. Flasks nos. 1 and 2 were immersed in boiling water for 5 min. before incubation.

Flask no.	Porphobilinogen added before incubation (mg.)	Porphobilinogen recovered after incubation (mg.)	Uroporphyrin (μg.)	Coproporphyrin (μg.)	Protoporphyrin (μg.)
1	0	0	0	0·16	4·10
2	1	0·59	0·71	0·36	2·90
3	0	0	0	0·29	6·25
4	1	0·52	0	0·26	2·75

coproporphyrin III injected parenterally into rats by Hoffbauer *et al.* (1953).

The absence of abnormal symptoms in any of the rats used in the present work confirms the results of the pharmacological study of porphobilinogen by Goldberg *et al.* (1954). It is of some interest that a small, though significant, rise of coproporphyrin III occurred in the urine of rats nos. 1, 2 and 9, besides some excretion of uroporphyrin III. This is in accordance with the demonstration by Falk *et al.* (1953) using a haemolysed chicken erythrocyte system, that porphobilinogen is a precursor of uroporphyrin, coproporphyrin and protoporphyrin.

The site of the excessive porphobilinogen formation in acute porphyria is of importance. Prunty (1945) showed that porphobilinogen is found in high concentration in the liver at autopsy of these cases, and this observation has been repeatedly confirmed. Thus Schmid, Schwartz & Watson (1954) include this disease in their 'porphyria hepatica' group. Porphobilinogen is also found in the kidney and urine and sometimes in the bile and plasma, but not in other tissues. In experimental animals drugged with sedormid or allyl*iso*propylacetamide, these same tissues also contain porphobilinogen and in the same relative concentrations. In fact Schmid & Schwartz (1952) refer to the experimental porphyria produced by sedormid as belonging to the 'hepatic type'. This suggested but did not prove, that in acute porphyria and in experimental porphyria of animals, porphobilinogen is formed in the liver. It did not exclude the possibility that porphobilinogen is formed at an extrahepatic site, e.g. the bone marrow, and is transported by the blood stream to the liver. The present experiments indicate that porphobilinogen is mainly excreted by glomerular filtration in the rat. Further, they show that porphobilinogen is not found in the liver after enteral or parenteral administration. The plasma porphobilinogen levels in these experiments were high, higher than those found in an attack of acute porphyria. In rat no. 9 this plasma level was maintained for 20 hr., a period which had been found sufficient to induce an experimental porphyria by means of allyl*iso*propylacetamide, with accumulation of porphobilinogen in the liver in rat no. 12. These facts would suggest that extrahepatic porphobilinogen formation in experimental porphyria in the rat is improbable. It is also of interest that there was no significant increase of either coproporphyrin or protoporphyrin in the livers of rats given porphobilinogen. In the livers of patients with acute porphyria and of animals with experimental porphyria such an increase is present.

There is some evidence that the mechanism of porphobilinogen excretion in the rat may be relevant to human acute porphyria. In this disease, the high porphobilinogen concentrations in the urine and the persistently low concentrations in the plasma, even at the height of an attack, suggest a rapid excretion of that substance. Furthermore, a sharp rise of porphobilinogen excretion in the urine often accompanies and may even precede the onset of clinical symptoms of porphyria, which would also suggest rapid elimination. Finally, acute porphyria urine, which is freshly passed and has not lain in the bladder for long, contains mainly porphobilinogen with only traces of coproporphyrin and uroporphyrin. This is the same pattern of porphobilinogen and porphyrin excretion as is found in the urine of a rat, injected parenterally with porphobilinogen. A greater conversion of porphobilinogen into porphyrins would be expected, if porphobilinogen were not rapidly eliminated in acute porphyria. The probability of such a rapid elimination would explain the absence of photosensitivity in this disease, since this symptom is caused by an excessive amount of formed porphyrins in skin. These findings would suggest that the renal excretion of porphobilinogen in the rat is similar to that in human acute porphyria.*

It has been pointed out that the porphobilinogen content of tissues in human acute porphyria and in experimental porphyria in animals is similar. This might suggest, in addition, that the reasoning put forward for a hepatic site of formation of porphobilinogen in the rat is valid for the human disease.

SUMMARY

1. Porphobilinogen has been administered parenterally and enterally to rats. After parenteral injection, porphobilinogen was rapidly and mainly excreted in the urine. Small amounts were excreted in the faeces. After enteral administration, only small amounts were slowly excreted in the urine. Most of it was passed unchanged in the faeces. The porphobilinogen excreted in urine was identical chromatographically with that administered.

2. A calculation of renal clearance of porphobilinogen in the rat suggests that porphobilinogen is mainly excreted by glomerular filtration without significant reabsorption.

3. A small but significant rise of coproporphyrin III, as well as the excretion of some uroporphyrin III, was noted in rat urine after parenteral injection of porphobilinogen. There was no significant change in faecal porphyrin excretion after enteral or parenteral administration of porphobilinogen.

4. Aerobic incubation *in vitro* at 37° of porphobilinogen with gastro-intestinal contents of a rat

* *Note added in proof.* Renal clearance studies recently done by the author in human acute and latent porphyria have shown that the endogenous porphobilinogen is excreted in man by glomerular filtration without significant reabsorption.

showed no evidence of conversion of porphobilinogen into porphyrins.

5. Porphobilinogen was not found in the liver when rats were killed shortly after its enteral or parenteral administration, nor was it found in the liver of a rat after subcutaneous injections of porphobilinogen every half-hour for 20 hr., during which time a high plasma porphobilinogen concentration was maintained. These findings suggest that the porphobilinogen found in the liver of a rat with experimental porphyria is in fact formed there and is not transported by the blood stream to the liver from an extrahepatic site.

6. The relevance of these results, obtained in the rat, to human acute porphyria, is discussed.

I would like to thank Professor C. Rimington for his considerable help and encouragement throughout this work, Dr E. I. B. Dresel and Dr S. E. Dicker for helpful criticism, and Miss B. C. Knight for technical assistance. Mr V. K. Asta kindly prepared the drawings.

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Preliminary Communication

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FATE OF PORPHOBILINOGEN IN THE RAT

RELATION TO ACUTE PORPHYRIA IN MAN

PORPHOBILINOGEN is excreted in the urine in large quantities in acute porphyria and is always found in the liver in fatal cases of this disease. In porphyria induced in animals by 'Sedormid' [1] or allyl-isopropyl-acetamide,[2 3] porphobilinogen is likewise found in the urine and liver. The isolation of porphobilinogen by Westall [4] has allowed a full study of the excretion of this substance when administered to rats. The results of these experiments may help in the understanding of the disease in man.

After parenteral injection porphobilinogen was rapidly and mainly excreted in the urine, being detectable there within 10 minutes of administration. Small amounts were excreted in the fæces. The porphobilinogen excreted in the urine was identical chromatographically with that administered. A small but significant rise of coproporphyrin III, as well as some uroporphyrin III, was noted in rat urine after parenteral injection of porphobilinogen. This confirms the demonstration by Falk, Dresel, and Rimington,[5] using a hæmolysed chicken-erythrocyte system, that porphobilinogen is a precursor of uroporphyrin, coproporphyrin, and protoporphyrin. Porphobilinogen given enterally is mainly excreted unchanged in the fæces; only traces are excreted in the urine.

The site of formation of porphobilinogen in acute porphyria is important. As already noted, it is always found in the liver in acute porphyria [6] and in experimental porphyria in animals [1 3]; and Watson and his school refer to both of these conditions as "porphyria hepatica." The possibility cannot be excluded, however, of an extrahepatic site of formation, from which porphobilinogen might be transported to the liver by the bloodstream. Because of the rapid renal elimination of porphobilinogen from the plasma, which has now been demonstrated, this seems unlikely. Further, porphobilinogen was not found in the liver when rats were killed shortly

1. Schmid, R., Schwartz, S. *Proc. Soc. exp. Biol., N.Y.* 1952, **81**, 685.
2. Goldberg, A. *Biochem. J.* 1954, **57**, ii.
3. Goldberg, A., Rimington, C. *Proc. roy. Soc.* B, 1954 (in the press).
4. Westall, R. G. *Nature, Lond.* 1952, **170**, 614.
5. Falk, J. E., Dresel, E. I. B., Rimington, C. *Ibid*, 1953, **172**, 292.
6. Schmid, R., Schwartz, S., Watson, C. J. *Arch. intern. Med.* 1954, **93**, 167.

after its enteral or parenteral administration, although the plasma at the time of death contained porphobilinogen.

Experimental porphyria was produced in a rat with allyl-isopropyl-acetamide within 20 hours of a single dose, the liver containing a high concentration of porphobilinogen (fig. 1, rat B). In order to mimic a possible extrahepatic site of porphobilinogen formation for this period, another rat (A) was injected subcutaneously with 1 mg. of porphobilinogen every half-hour for 20 hours. Fig. 2 shows that a high plasma-porphobilinogen level was maintained throughout this time. Rat A was killed 15 minutes after the final injection ; the liver did not contain porphobilinogen. This suggests that extra hepatic porphobilinogen formation in experimental porphyria in the rat is improbable and points to the liver as the site of its formation in that condition.

The plasma-porphobilinogen level throughout the latter 10 hours of the 20-hour experiment (fig. 2) was

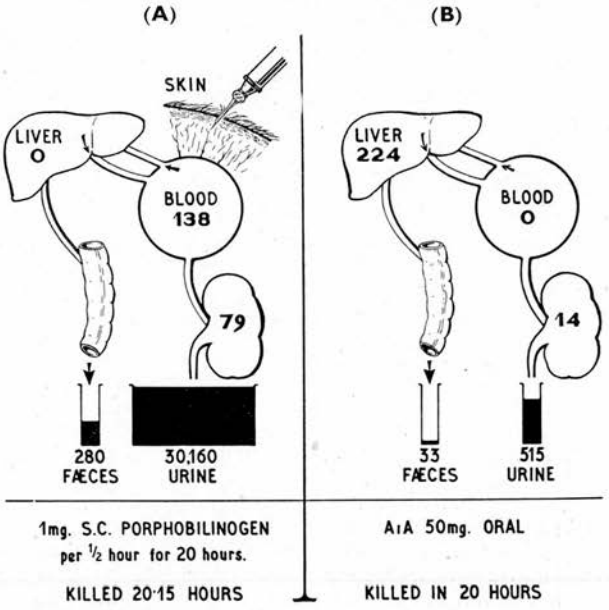

Fig. 1—Porphobilinogen (μg.) in tissues of rats A and B. Rat A received repeated subcutaneous (S.C.) injections of porphobilinogen. Rat B was given a single oral dose of allyl-isopropyl-acetamide (A.I.A.). No porphobilinogen has accumulated in the liver of rat A, despite maintained high blood-level, whereas the liver of rat B contains much porphobilinogen.

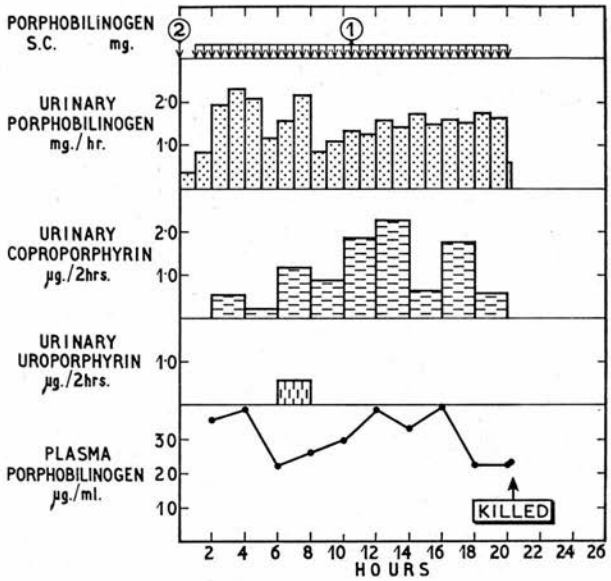

Fig. 2— Rat A. Repeated subcutaneous injections of porphobilinogen for 20 hours. Urinary porphobilinogen and porphyrin excretion and plasma-porphobilinogen levels.

31∓7·4 µg. per ml., while porphobilinogen was excreted in the urine at a rate of 1·530∓0·151 mg. per hour. From this it has been calculated that the renal clearance for porphobilinogen was 0·82 ml. per min. in this rat or 0·55 ml. per min. per 100 g. of body-weight. This figure agrees closely with inulin clearance found in the rat [7] and therefore suggests that porphobilinogen is mainly filtered by the glomeruli. The rats in all these experiments had no abnormal symptoms after administration of porphobilinogen. This confirms recent pharmacological studies on porphobilinogen.[8]

There is some evidence that the mechanism of porphobilinogen excretion in the rat may be relevant to acute porphyria in man. In this disease the high porphobilinogen concentrations in the urine and the persistently low concentrations in the plasma, even at the height of an attack, suggest a rapid excretion of that substance. Furthermore, a sharp rise of porphobilinogen excretion in the urine often accompanies, and may even precede,

7. Smith, H. W. The Kidney: Structure and Function in Health and Disease. New York, 1951: p. 531.
8. Goldberg, A., Paton, W. D. M. Thompson, J. W. *Brit. J. Pharmacol.* 1954, **9**, 91.

the onset of clinical symptoms, which also suggests rapid elimination associated with an exacerbation of the disorder. Finally, in acute porphyria, urine which is freshly passed and has not lain long in the bladder contains mainly porphobilinogen with only traces of coproporphyrin and uroporphyrin. This is the same pattern of porphobilinogen and porphyrin excretion as is found in the urine of a rat injected parenterally with porphobilinogen. In acute porphyria greater conversion of porphobilinogen to porphyrins would be expected if porphobilinogen were not rapidly eliminated. Such a rapid elimination would explain the absence in this disease of photosensitivity, which is caused by an excessive amount of formed porphyrins in skin. These findings suggest that the renal excretion of porphobilinogen in the rat is similar to that in acute porphyria in man.

As has been pointed out, the porphobilinogen content of tissues in acute porphyria in man is similar to that in experimental porphyria in animals[13]; which suggests that the reasoning put forward for a hepatic site of porphobilinogen formation in the rat is valid for the human disease.

We wish to thank Dr. E. I. B. Dresel and Dr. S. E. Dicker for helpful criticism and Miss B. C. Knight for technical assistance. A detailed account of this work will shortly be published elsewhere by one of us (A. G.).

A. GOLDBERG
M.B. Edin., M.R.C.P., F.R.F.P.S.

C. RIMINGTON
M.A., Ph.D. Camb., D.Sc. Lond., F.R.S.

Department of Chemical Pathology, University College Hospital Medical School, London

The Lancet Office,
7, Adam Street, Adelphi, London, W.C.2

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HEREDITARY COPROPORPHYRIA

BY

H. BERGER, M.D.

Universitätskinderklinik, Jennerspital, Berne, Switzerland

AND

A. GOLDBERG, M.B., Ch.B., F.R.F.P.S., M.R.C.P.

Department of Chemical Pathology, University College Hospital Medical School, London

In 1936 Dobriner reported a case in which a woman excreted large amounts of coproporphyrin (probably series III) and traces of uroporphyrin in the urine, and also large amounts of coproporphyrin (series I and III) and protoporphyrin in the stool. Although this woman was then a patient in a psychiatric ward, there were no apparent symptoms associated with the abnormal porphyrin excretion. Watson *et al.* (1949) described the cases of two men who excreted large amounts of coproporphyrin III in the urine and stools, unaccompanied by symptoms. They stated that this condition, which they called "idiopathic coproporphyrinuria," represented an "inborn error of metabolism," although they could not find evidence for any hereditary association of the abnormality.

The present paper describes the occurrence of a similar pigment disturbance in a Swiss boy, in his mother and father (who are first cousins), and also in his paternal aunt. The boy excretes much higher quantities of coproporphyrin in his urine and faeces than the others and also passes traces of uroporphyrin I in his urine. He has suffered from rickets, noted for the first time at 3½ years, as well as from riboflavine deficiency. An unusual amino-acid excretion pattern was obtained by paper chromatography in three of these four subjects. The term "hereditary coproporphyria" has been used for this condition, because of the genetic significance of these findings and because the porphyrins are excreted in the faeces as well as in the urine, principally in the faeces.

Methods

Porphyrins.—Quantitative determinations of porphyrins and porphobilinogen in the urine and stools were carried

out as in a previous study (Goldberg, 1954). Since initial investigation had shown that the excess porphyrins were entirely ether-soluble, the isolation and identification were done as under "ether-soluble porphyrins."

Urinary amino-acids were identified by paper chromatography (Dent, 1951).

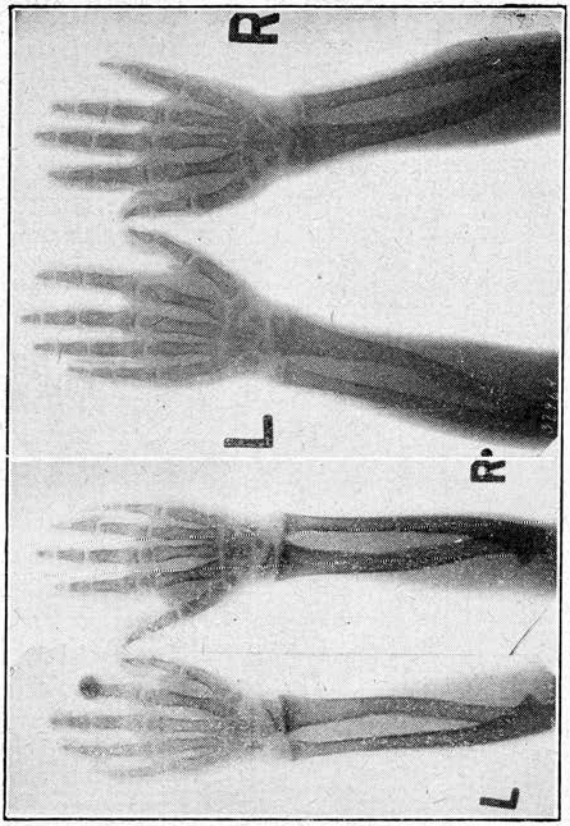
Riboflavine determinations were carried out by means of microbiological assay, using *Lactobacillus casei,* or fluorimetrically.

Case Report

This patient was born in 1944 after a normal labour. His mother had hyperemesis during pregnancy. Birth weight, 3 kg. He was breast-fed until the age of 7 months, then was given breast milk and vegetables until 1 year, when he was put on a mixed diet. His first tooth erupted at 1 year and he completed his primary dentition by the end of his second year. He started walking at 1½ years and spoke his first word at 2. About the age of 1½ years he had a generalized skin rash, which disappeared when milk was discontinued. About this time he also had diarrhoea, which was treated in the out-patient department of the Jenner-Kinderspital, Berne, Switzerland. At 3½ years he had a severe middle-ear infection, which later was complicated by a throat and lung infection. He recovered from this, but one month later developed gastro-enteritis with marked dehydration. At this time his family doctor noted that he had rickets and gave him calcium and vitamin preparations without benefit. At 4 years his mother observed that some of his nails were cracked and heaped up and that there was "eczema" between his fingers, where infection was common. There was also some cracking and irritation of the buccal mucous membrane and at the angles of the mouth. His mother believed that when he drank milk his mouth and hands got worse. His skin had never been affected adversely by the sun.

On September 22, 1952, he was admitted to the Jenner-Kinderspital under the care of Professor Ed. Glanzmann because of further attacks of diarrhoea and also for treatment of his rickets, mouth, and nails. It was during this admission that an investigation of the urine disclosed the incidental presence of excessive porphyrins.

Physical examination showed a normally intelligent boy with rickets (Fig. 1), undersized and underweight for his age. He was 108 cm. in height, 18.5 kg. in weight, while the standard height and weight for his age were 123 cm. and 24.4 kg. respectively. There was slight hypertelorism. The thorax was funnel-shaped, with a marked rachitic rosary. There was thickening of the epiphysial ends of the long bones, shortening and bowing of the femur, and antero-lateral bending of the tibiae. There was marked cheilosis and the tongue was red, shiny, and without



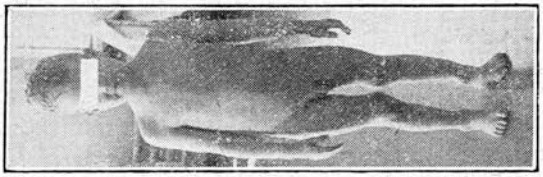


FIG. 1 FIG. 2 FIG. 3

FIG. 1.—The patient, showing presence of rickets. FIG. 2.—Forearms and hands of patient before treatment.
FIG. 3.—Forearms and hands after one year of treatment.

papillae. His skin was generally dry, scaly, and hyperkeratotic, and there was also hyperkeratosis of the nails. Examination of his eyes showed a bilateral granular and flocculent cataract, denser centrally and finer more peripherally. He was hypermetropic. There was no further abnormality on physical examination ; in particular, the liver and spleen were not enlarged. His blood pressure was 125/60. Chvostek's and Trousseau's signs were negative.

Tests

Peripheral Blood.—Hb 78% ; R.B.C., 4,000,000 per c.mm., reticulocytes, 0.6% ; platelets, 150,000 per c.mm. W.B.C., 5,000 per c.mm. (neutrophil polymorphs 57%, lymphocytes 29.5%, monocytes 8.5%, eosinophils 4%, basophils 1%). Bleeding, clotting, and prothrombin times were normal. E.S.R. (Westergren), 18 mm. in one hour. Bone-marrow examination, normal. Serum calcium, 9.7 mg./100 ml. ; serum phosphorus, 2.86 mg./100 ml. ; plasma phosphatase, 16 units (Bodansky). Total protein, 7.50 g.% (albumin 4.90 g., globulin 2.60 g.). Total cholesterol, 134 mg./100 ml. Plasma alkali reserve, 44 vols.%. Blood urea, 28.8 mg./ 100 ml. W.R. negative. Moro, Pirquet, and Mantoux reactions (1/10,000 to 1/100), negative.

Urine occasionally showed traces of protein. A water concentration and dilution test was normal (S.G. 1026 to 1000). Porphyrin investigations are reported below.

Liver Function Tests.—Takata–Ara and cadmium, normal ; Weltmann, 0.35% $CaCl_2$ (normal, 0.4–0.5) ; serum bilirubin, 0.83 mg./100 ml. A fat balance was not done.

Progress

The patient was treated with large doses of vitamin D orally and B_2 (riboflavine) orally or intravenously. During the period September 22 to December 24 (94 days) he received a total of 1,458,000 I.U. of vitamin D and 4,675 mg. of riboflavine, with additional vitamins A, C, and B complex. The general condition of the child improved. His rickets improved greatly and his mouth and tongue were better. During this period of treatment several serum and urinary determinations were carried out on the patient and on a control of approximately the same age and weight. The serum and urinary riboflavine levels rose from 3.07 and 62.2 μg./100 ml. on September 24 to 97.0 and 4,062 μg./ 100 ml. respectively on October 30. The initial serum and urinary riboflavine levels were well below those of the control, which were 25–32 and 164–784 μg./100 ml. respectively. He was readmitted for further investigation in September, 1953. In the 15 months since his previous admission he had grown to 114 cm. in height. His measurements were: crown to pubis, 58 cm. ; pubis to heel, 56 cm. ; arm span 116 cm. He was still slightly bow-legged, but the *x*-ray film of his hands and forearms showed marked improvement of his rickets (Figs. 2 and 3). There was also considerable improvement of the cataract. Marked porphyrin fluorescence was noted in his upper incisor and molar teeth, but not in his lower incisors. There was no fluorescence elsewhere, except in the circumanal region, and this was due to faecal contamination. His blood plasma did not fluoresce in ultra-violet light. His urine and faeces still contained very large amounts of porphyrin (Table I).

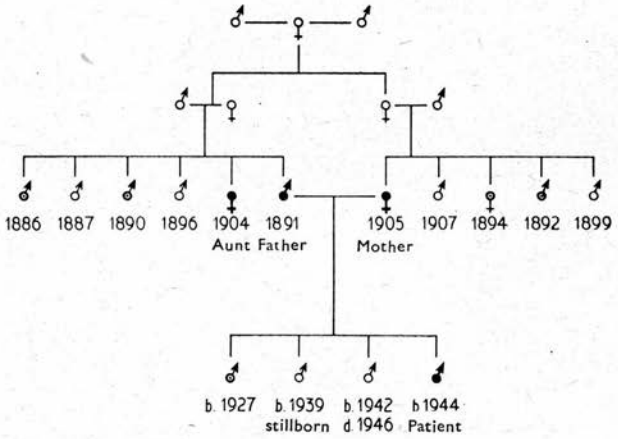
TABLE I.—*Quantitative Determinations. Urinary and Faecal Porphyrins*

	Urine			Faeces		
	Porphobilinogen	Uroporphyrin	Coproporphyrin (μg./l.)	Uroporphyrin	Coproporphyrin (μg./g. Dry Weight)	Protoporphyrin (μg./g. Dry Weight)
Normal (maximal)	Negative	Negative	100	Negative	15	30
Patient ..	,,	Negative*	5,719	,,	2,082	56·8
Mother ..	,,	Negative	500	,,	525	23
Father ..	,,	,,	775	,,	862	44
Aunt ..	,,	,,	295			

* Small amounts detected by chromatographic methods.

Family History

The patient's mother and father are aged 48 and 62 years respectively. Both are healthy and have never had any skin disease, deformity, or growth abnormality. The father is a farmer, and the general diet of the family is undoubtedly satisfactory. The patient has one living brother, aged 26 years, who is normally grown and healthy. Two other brothers are dead. One was stillborn at the sixth month of pregnancy, and the other died at 4 years from pneumonia. The latter was said to have had a brain injury during his birth and suffered from recurrent epilepsy (grand



Family tree. The mothers of the patient's father and mother were stepsisters.

○ Urine and stool not examined.

⊙ Urine only examined. Normal coproporphyrin excretion.

● Increased porphyrin output in stool and urine. (Stool of the aunt not examined.)

FIG. 4

mal). The patient's paternal aunt, aged 59, is alive and well. There is no relevant disease in other relatives, so far as could be ascertained. The family tree is reproduced in Fig. 4.

Further Investigations

Porphyrins.—Tables I and II summarize the results of the urinary and faecal porphyrin investigations. There are marked increases in the coproporphyrin III excretion in the four cases. In the mother there is a fivefold and 35-fold increase of urinary and faecal coproporphyrin respectively; in the father an eightfold and 64-fold increase above maximal normal values. The urinary coproporphyrin of the aunt is increased threefold. The urinary and faecal coproporphyrins in the child are particularly high, and are more than 50 and 100 times the maximal normal values respectively. Uroporphyrin I was isolated from his urine in crystalline form as its methyl ester. A porphyrin, which behaved consistently as pentacarboxylic by paper chromatographic methods, was isolated. Although its α band in chloroform and its behaviour both as an ester and as the free porphyrin were distinctive, yet its crystalline appearance and melting-point were very similar to those of coproporphyrin III. Evidence was also obtained by alumina and paper chromatography of the presence of a pentacarboxylic porphyrin in the urine of the mother and aunt, and of a hexacarboxylic porphyrin in the stool of the patient and in the stool and urine of his father.

The urinary amino-acids of the patient and his mother and father, investigated by paper chromatography, showed in each case a "super-glycine pattern." This term is being currently used by Dent (private communication) and his collaborators to describe the pattern shown on a standard urine chromatogram (280 μg. total N) when the amino-acid excretion is comparatively normal except for a large excess of glycine. Those workers have encountered several individuals who appear to excrete amino-acids constantly in this manner. Its pathological significance is still under investigation. Urine from the aunt or other members of the family could not be obtained for this investigation.

The Sulkowitch test in the urines of the patient, mother, and father showed each to have a very high calcium concentration. In the case of the patient the urine was given this test before and after vitamin D treatment had begun.

Discussion

The condition in the four members of the family described above is similar, as regards the disturbances of porphyrin metabolism, to that described by Dobriner (1936) and Watson *et al.* (1949). The pigment excretion in Dobriner's case, in which traces of uroporphyrin were found in the urine along with greatly increased ether-soluble por-

TABLE II.—*Chemical Identification of Porphyrins in Urine and Faeces*

		Free Porphyrin		Porphyrin Ester (Paper Chromatography)	M.P. of Crystals (°C.)	Summary
		No. of COOH Groups	α Band in $CHCl_3$ (mμ)			
Patient	Urine	4	621·5	Copro III	140–143, 166–167	Coproporphyrin III
		5	623·0	Between copro I and III	154, 172–178	Pentacarboxylic porphyrin
		8	625·3	Mainly uroporphyrin series I	291	Uroporphyrin I
	Faeces	4	621·3	Copro III	142, 162–166	Coproporphyrin III
		6	623·0			Hexacarboxylic porphyrin
Mother	Urine	4	621·5	Copro III	138–142, 164–168	Coproporphyrin III
		5	622·9			Pentacarboxylic porphyrin
	Faeces	4	621·5	,, ,,	141–143, 164–168	Coproporphyrin III
Father	Urine	4	621·6	,, ,,	143	Coproporphyrin III
		6	622·9			
		4	621·5	,, ,,	140, 164–166	Coproporphyrin III
	Faeces	6	623·3			Hexacarboxylic porphyrin
Aunt	Urine	4				Mainly coproporphyrin (trace pentacarboxylic porphyrin)
		Trace				
		5				

phyrins, would seem to correspond closely to that of our patient. The demonstration, however, of this abnormal porphyrin excretion as a hereditary trait, less severe in the parents and the paternal aunt, and much more severe in the child, gives the present group of cases a significance which is important. The diseases of porphyrin metabolism have long been described as " inborn errors of metabolism " (Garrod). Congenital porphyria is accepted as being transferred as a Mendelian recessive, while there is strong evidence that acute porphyria occurs as a Mendelian dominant. The inheritance of porphyria cutanea tarda is still in doubt. The condition described above neither clinically nor biochemically corresponds exactly to any of these three diseases. The evidence presented suggests that the genetic pattern of the dyscrasia is heterozygous in the parents and paternal aunt, and is homozygous in the child.

The association of rickets and riboflavine deficiency with excessive porphyrin excretion in our patient is of interest. Rickets has never hitherto been reported in association with a porphyrin disorder. The aetiology of the rickets is a matter of question. The bony deformities were noted only at the age of 3½ years. Since the age of 1½ years the mother considered that milk aggravated his skin condition. It is therefore possible that the withholding of milk over a long period contributed to the rickets in a patient whose disease predisposed him to it. On the other hand, renal acidosis or some defect in the renal tubular reabsorptive mechanism, or even steatorrhoea, has not been fully excluded. The fact that the improvement in the bone condition was not accompanied by any marked change in the porphyrin excretion suggests that the association of the rickets and excessive porphyrin excretion is not a close one.

Some relationship may exist between the porphyrin disorder and a predisposition to riboflavine deficiency, since an accidental association of these two excessively rare disorders is most unlikely. It is of interest that Stich (1952) has suggested that riboflavine acts as a regulator of biological porphyrin synthesis, directing it to the formation of the series III porphyrin isomers rather than series I, which, he claims, accumulate excessively when this vitamin is deficient. He based this hypothesis on experimental studies with yeast cultures and supported it with apparently successful therapeutic trials of riboflavine in cases of porphyria. This form of therapy had also previously been advocated by Vannotti (1937), and has also been used by Weingarten (1950) and Lups and Van Dijk (1950). The evidence obtained so far in the present case does not lend support to Stich's hypothesis, since most of the excessive porphyrin excreted is of the III series and only a small amount is excreted as uroporphyrin I. Since the completion of the present studies Antener and Berger (1955) have found that after the administration of 60 mg. of riboflavine daily to our patient, and after periods without riboflavine,

the urinary excretions of porphyrin and riboflavine showed an inverse relationship. Thus high levels of urinary porphyrin excretion were associated with low levels of riboflavine excretion, and vice versa. The interpretation of these findings and their possible relation to Stich's hypothesis will be discussed by these authors.

The absence of symptoms, such as abdominal colic or constipation, in every case supports the findings of Goldberg, Paton, and Thompson (1954), that the known porphyrins have no pharmacological action. Our patient has shown no sign of photosensitivity, despite a high daily excretion of coproporphyrin. It has been suggested (Fischer and Zerweck, 1924) that the phototoxic action of the porphyrins is directly proportional to the number of carboxyl groups in the porphyrin molecule ; thus coproporphyrin, the main excretion product in this case, would be less active in this respect than uroporphyrin, which is excreted in congenital porphyria and generally in porphyria cutanea tarda. The absence of plasma fluorescence would also suggest a very rapid clearance of coproporphyrin from the circulation, which would prevent an effective concentration of photosensitizing porphyrin in the skin. Watson *et al.* (1949) failed to find raised blood porphyrin levels in their cases. Both these factors—the presence of coproporphyrin rather than uroporphyrin and the efficient elimination of the porphyrin mainly by the faeces—might explain the lack of photosensitization.

The mechanism of the excessive porphyrin production is of interest. In the present case the absence of anaemia, despite the loss of approximately 50 mg. of coproporphyrin daily for several years, would suggest an overproduction of these pigments (Goldberg and Rimington, 1955). The porphyrin is greatly overproduced in the homozygote (the boy), only moderately so in the heterozygotes. This quantitative regulation of the pigments is thus apparently controlled genetically. The main route of excretion of the excess coproporphyrin is via the faeces, which renders unlikely any " renal leak " mechanism. The site of production of these excess pigments is probably the liver or the bone marrow, since one or other of these tissues contains large quantities of porphyrins in the main porphyria diseases. Further work is required to clarify this point. Hoffbauer, Watson, and Schwartz (1953) found that coproporphyrin III, given orally to the rat, was not absorbed from the gut. If this finding is applicable to the human it would make unlikely any theory of gut synthesis of coproporphyrin in this condition. No trial with chlortetracycline has been made so far.

Summary

Four cases of coproporphyria have been described in a single family—a boy aged 10, his mother and father

(first cousins), and his paternal aunt. The symptoms of porphyria, such as photosensitivity, abdominal colic, constipation, and paralysis, were absent. The adults excreted moderately high quantities of coproporphyrin III in the urine and stool, whereas the boy excreted very large quantities of coproporphyrin III in the urine and stool, with a trace of uroporphyrin I in the urine. Intermediate porphyrins were also identified—a pentacarboxylic porphyrin in some urines and a hexacarboxylic porphyrin in some stools. In all cases examined the main route of porphyrin excretion was via the stool. The genetic significance of this hereditary porphyrin disorder has been discussed.

The boy also suffered from rickets and riboflavine deficiency, both of which were improved by treatment.

Both the boy and his parents had an amino-aciduria of somewhat unusual character, which is discussed.

The significance of the symptomless nature of this condition and the mechanism of its production are considered.

We wish to thank Professor C. Rimington, F.R.S., and Professor Ed. Glanzmann for their help and encouragement in this work. Dr. C. E. Dent gave advice on amino-acid chromatography and valuable criticism. Dr. H. Harris advised on the genetical aspect of the investigation. We are grateful to Dr. I. Antener, Leiterin des Kontroll-Laboratoriums der Fa. Nestle, Vevey, Switzerland, for the riboflavine assays and to Frl. R. Wermuth and Miss A. Benson for technical assistance. A visit by one of us (A. G.) to Berne was assisted by a grant from the Rockefeller Research Fund of University College Hospital Medical School.

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THE NEUROPATHOLOGY OF ACUTE PORPHYRIA

JAMES B. GIBSON * and A. GOLDBERG

Department of Pathology, University and Western Infirmary, Glasgow and Department of Chemical Pathology, University College Hospital Medical School, London

(PLATES CXXXIX-CXLII)

THE gravest clinical features of the intermittent attacks that characterise *acute porphyria* are the neurological manifestations, first described by Ranking and Pardington (1890). Similar manifestations sometimes occur in *porphyria cutanea tarda* but they are not found in congenital porphyria. Purely paralytic and purely abdominal forms of *acute porphyria* were defined by Waldenström (1937), together with a combined or classical form, a terminal comatose form and a latent form. This division between abdominal and paralytic forms can be justified on prognostic grounds, for attacks with nervous symptoms have a much higher mortality than those with abdominal symptoms only (Baker and Watson, 1945 ; Vannotti, 1954). Abdominal symptoms, however, are a common prelude to a classical attack and, as Waldenström admits, the purely paralytic form is very rare. The clinical forms designated by Waldenström, with the exception of the latent, therefore probably differ little in essence. Persons with latent porphyria are susceptible to attacks, and innumerable precipitating causes of these have been cited in the literature. Many attacks are brought on, or made worse, by barbiturate medication, and in recent years it has not been customary to divide acute porphyria into idiopathic and toxic varieties. The disease shows a strong familial tendency.

The excretion of a monopyrrolic chromogen, porphobilinogen, is the characteristic biochemical abnormality in acute porphyria, whether in the latent or overt phase, and indicates a profound disturbance of pyrrole pigment metabolism. Porphobilinogen is not present in congenital porphyria nor is it yet known with certainty to occur in any other disease, except during those attacks of porphyria cutanea tarda which resemble acute porphyria in type (Schmid *et al.*, 1954 ; Watson, 1954). Uroporphyrin is now considered of secondary importance in the biochemical disturbance.

Berg (1945) and Vannotti (1954) take the view that the important clinical symptoms of acute porphyria have a functional but no morbid anatomical basis. While this may be true of the earlier stages of an attack, demyelination has been demonstrated, especially in peripheral

* Present address : Institute of Pathology, Queen's University, Belfast.

nerves, by many different investigators. It is patchy, widely disseminated and without predilection for any special nerve, so that a random section may give little hint of what is in fact extensive damage. The only lesions recognisable on naked-eye examination are the chronic ones resulting from repeated attacks. Previous histopathological reports, which have mainly been concerned with single cases, have demonstrated that the nervous system bears the brunt of an attack of acute porphyria, and we have confined our observations in this paper to that system. The results of this investigation in 5 fatal cases emphasise the frequency with which demyelination occurs and support the view that the neural lesions are chiefly responsible for the clinical manifestations of an attack.

Material and methods

Nervous and other tissues and clinical details of 5 fatal cases have generously been made available to us from a number of sources throughout the country. Post-mortem urinary uroporphyrins were estimated in a Beckman spectrophotometer (Model DU) by the method of Rimington and Sveinsson (1950) after heating the urine for 20 minutes with a *p*H 4·2 acetate buffer.

The principal stains and staining methods used for tissues were cresyl-violet, phosphotungstic acid-hæmatoxylin, sudan IV, Holmes's axon stain, and the Loyez, Spielmeyer and Bielschowsky-Gross methods. Unstained frozen sections of the spinal cord of cases 3-5 were examined microscopically in ultraviolet light from a mercury vapour lamp and viewed through a red filter (Wratten series 25) for porphyrin fluorescence. The tissues had all been preserved for long periods in formalin. Control sections from random autopsies were similarly treated and examined.

Case histories

Case 1. A 33-year-old lorry-driver was admitted to hospital on 27.2.53 complaining of muscular pains for one week and of numbness of the thighs and genitalia. He was constipated. He had taken no barbiturates before admission. The patient had had an attack of rheumatic pains in 1944 and at that time had passed red urine. He was agitated and showed muscular incoordination and nystagmus to the left. Perception of pain and light touch was diminished in the bathing-trunks area. B.P. 130/70 mm. Hg.

Quinalbarbitone, gr. iii, was given as a sedative on each of his first 6 nights in hospital. He gradually weakened, lost the power of swallowing and had difficulty in coughing, developed complete paralysis of the arms and became incontinent. The urine was dark red and contained porphobilinogen and uroporphyrin series III. Death on 8.3.53 from respiratory failure. There was no family history of note. Post-mortem urinary uroporphyrin 12 mg. per litre.

Case 2. A 52-year-old housewife was admitted to hospital on 4.6.53 on account of morning vomiting for 6 weeks. She was hypertensive (B.P. 250/140 mm. Hg.) and had been for some time. Twelve days later she complained of abdominal pain. She subsequently received quinalbarbitone, gr. vi, in a hypertension-sedation test. Next day, 18.6.53, she behaved strangely and the test was repeated. Pains were severe in abdomen and also in upper thighs. She became constipated and at times incontinent of urine. On 27.6.53 the urine was dark red and contained porphobilinogen and uroporphyrin series III. The patient lost pin-prick sensation, complained of generalised numbness and developed a flaccid quadriparesis. B.P. fell to 150/110 mm. Hg. She became dyspnœic and died from acute heart failure on 30.6.53. There was no family history of note. Post-mortem urinary uroporphyrin 38·85 mg. per litre.

THE NEUROPATHOLOGY OF ACUTE PORPHYRIA

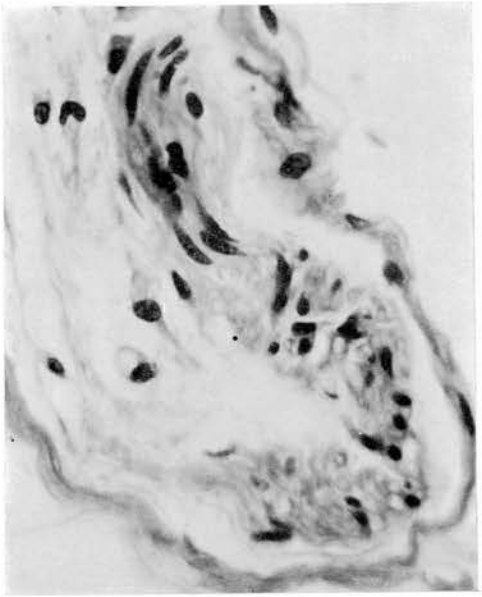

FIG. 1.—Case 1. Femoral nerve bundle, showing œdema and occasional vacuolated macrophages. Hæmatoxylin and eosin. ×500.

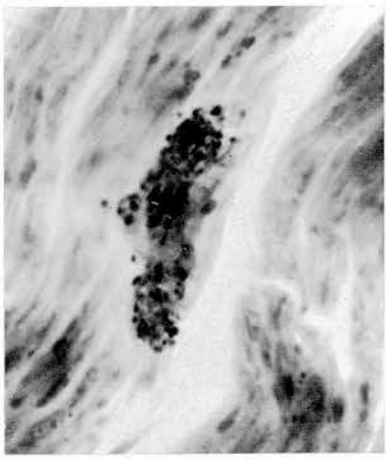

FIG. 2.—Case 4. Right abdominal sympathetic chain: lipoid granules in a myelinophagic cell. Sudan IV. ×1000.

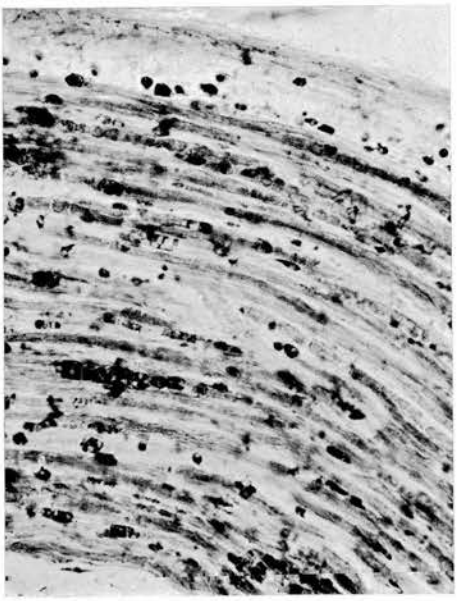

FIG. 3.—Case 4. Right phrenic nerve undergoing demyelination, with phagocytosis of released lipoid. Free macrophages are seen in œdematous spaces beneath the endoneurium. Sudan IV. ×120.

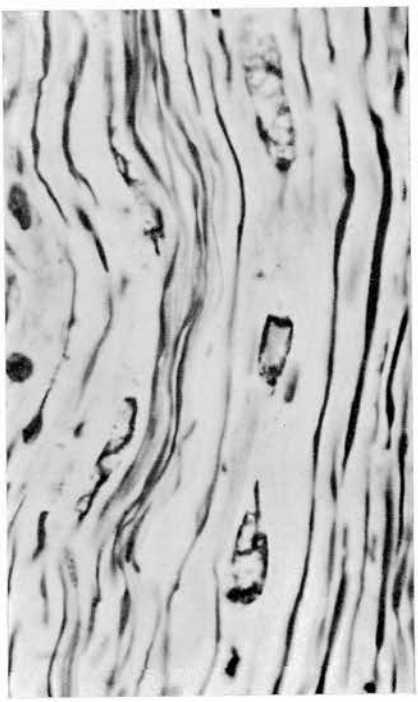

FIG. 4.—Case 1. Sciatic nerve; 2 axons have disintegrated into granular globules, but most are intact. Holmes's axon stain. ×420.

THE NEUROPATHOLOGY OF ACUTE PORPHYRIA

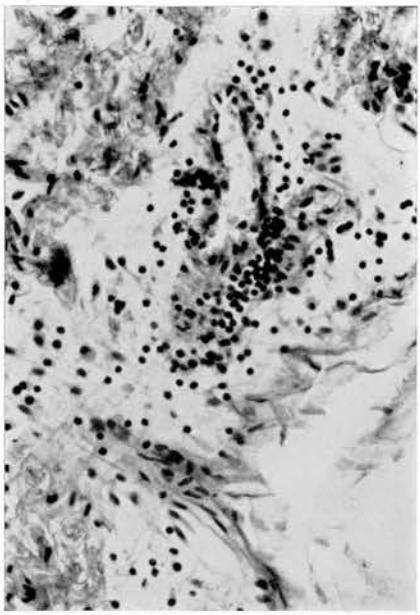

FIG. 5.—Case 4. Right phrenic nerve. There is cellular infiltration of an œdematous space under the perineurium. Lymphocytes surround a small vein beside an endoneurial septum. H. and E. ×200.

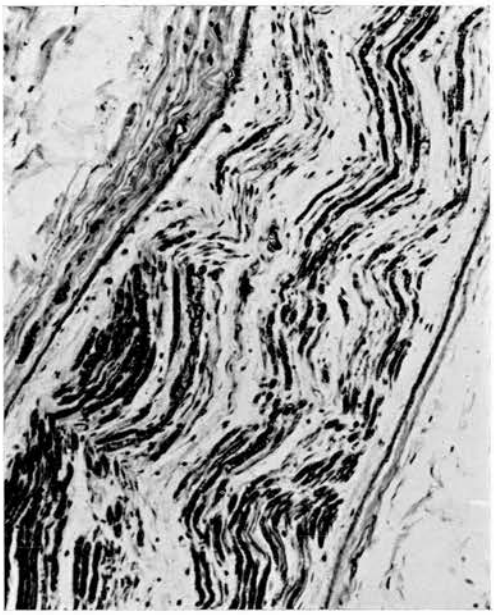

FIG. 6.—Case 3. Left musculo-cutaneous nerve bundle in which myelin sheaths are reduced in number and persisting myelin is degenerating. The bundle is œdematous and the perineurium, on the left, is slightly thickened. Loyez. ×100.

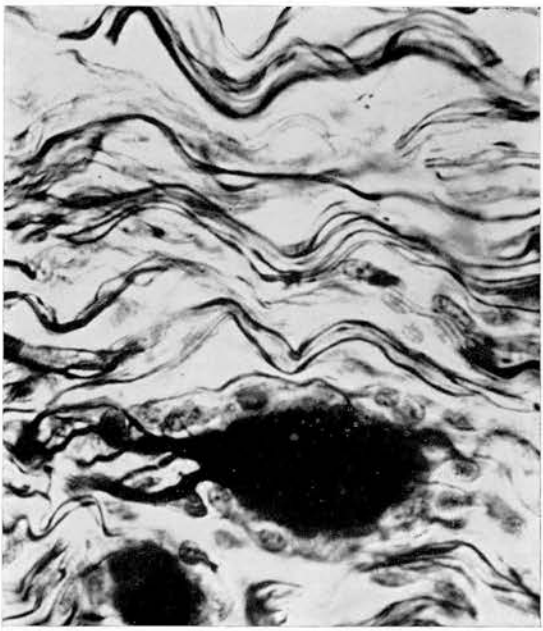

FIG. 7.—Case 4. Left abdominal sympathetic chain ; normal axons and nerve cell. Bielschowsky-Gross. ×500.

Case 3. An 18-year-old apprentice butcher was admitted to hospital on 4.9.53 on account of headache and pains in the back and limbs. There was no history of taking barbiturates at any time. He had had several attacks of abdominal and leg pains in the past, the first being in February 1952. In some of these he had developed transient hypertension (B.P. 170/100 ; 190/140 mm. Hg.) and mental disorientation. During all attacks, porphobilinogen and uroporphyrin had been identified in the urine. During his final hospital admission he developed tremor and weakness of his arms and legs and became disorientated. The weakness became worse and involved respiratory muscles. He received cortisone from 29.10.53 to 20.11.53 (total 8·5 g.) and from 7·12 to 11·12 (total 600 mg.). He died from bronchopneumonia on 14.12.53. His sister suffers from acute porphyria. Post-mortem urinary uroporphyrin 77 mg. per litre.

Case 4. A 21-year-old woman was well until 10 weeks before her death when she first complained of lower abdominal pain, constipation, vomiting, dysuria and amenorrhœa. At that time the plantar responses were extensor. She lost 1 stone in weight in the first 4 weeks of her illness, during which she was given phenobarbitone, gr. ½ t.d.s. Two weeks before death she developed weakness of her arms and legs and began to behave hysterically. She was admitted to a mental hospital on 4.3.52, where she developed flaccid quadriplegia with difficulty in swallowing and breathing. B.P. was 100/76 mm. Hg. The urine contained porphobilinogen and uroporphyrin series III. She died in a Drinker respirator from respiratory failure on 11.3.52. There was no family history of note. Post-mortem urinary uroporphyrin 81·5 mg. per litre.

Case 5. A woman aged 53 was admitted to hospital on 19.10.53 for total hysterectomy on account of uterine fibromyomata. She had suffered from menorrhagia and had been losing weight for 8 months. She had taken sedative doses of sodium amytal, soluble phenobaritone and quinalbarbitone on various occasions, and thiopentone, 150 mg., at the time of the hysterectomy on 21.10.53. After operation her abdomen became distended and her wound burst open. On 3.11.53 she passed red urine containing uroporphyrin ; subsequently peripheral neuritis developed and became advanced. B.P .180/110 mm. Hg. She died from bronchopneumonia on 10.11.53.

The patient's sister had had an attack of acute porphyria one month previously and this sister's daughter died in a mental hospital with psychotic symptoms which began after an attack of acute porphyria.

Histology

Peripheral nerves. Acute lesions in various stages of evolution are demonstrable in peripheral nerves in all cases. The lesions are multiple, segmental and scattered at intervals along the affected nerves, and no particular nerve is selected for attack. The lesions are early and of minor degree in cases 2 and 5 and well established and severe in case 3. In case 3 both chronic and acute changes are present. In the least affected nerve bundles, *e.g.*, the sciatic nerves in cases 2 and 5, the neuropathy is recognisable in paraffin sections by the presence of œdema of the bundles and vacuolated macrophages (fig. 1). In frozen sections, sudanophilic granules are seen in myelinophagic cells (fig. 2). In more severely affected nerve segments, the whole course of demyelination can be traced, beginning with fragmentation of the myelin sheaths and their breakdown into unnatural forms. This is followed by phagocytosis of fractions of the degenerate myelin and by the development of œdema, giving rise to clear spaces under

the perineurium and round the endoneurial septa, in which free macrophages are gathered in moderately large numbers (fig. 3). Free macrophages are more frequent than Wallerian ovoids, even in these severely damaged nerves. The endothelium of blood capillaries is swollen and proliferated. Sometimes axon changes are absent or insignificant, in other instances there are striking irregularities in calibre and the axons may be broken up into granular fragments and globules (fig. 4). In general, axon damage parallels myelin degeneration but is neither so extensive nor so frequent. Schwann and neurilemmal cells are increased in prominence and number. In the most affected patches an infiltration of lymphocytes is seen round the small blood-vessels of the nerve (fig. 5). In some of the nerves of the left brachial plexus in case 3, in which repeated attacks had occurred, the number of axons and of their myelin sheaths is much reduced (fig. 6). Schwann cells are found throughout these nerves and there is a little increase in collagen, especially in the perineurium. There is also a moderate degree of acute demyelination and accompanying cellular reaction.

Autonomic nervous system. Acute demyelination is seen in the vagus nerves in cases 2 and 4 and in fibres of the sympathetic chain in case 4 (fig. 2), but axons are intact (fig. 7). There is slight peripheral vacuolation of ganglion cells of the chain in case 3, chromatolysis in case 4 and a slight increase in perineuronal cells in case 2.

Spinal posterior-root ganglia. The changes here are, in general, slight and inconstant. As elsewhere in the nervous system many of the ganglion cells contain a little more lipofuscin than usual.

Spinal cord. Chromatolysis of the anterior-horn cells is the commonest change in the central nervous system and is pronounced in all cases. There is a tendency for one level or another of the cord to be affected most severely in individual cases, but no level is attacked preferentially. Even in the worst areas, unaffected cells may be seen alongside severely chromatolytic ones (fig. 8). The earliest chromatolysis is perinuclear. Later it is generalised and accompanied by granular change in the neurofibrils and dislocation of the nucleus. In some instances the anterior-horn cells are pyknotic or poorly defined, but the only consistent changes, apart from chromatolysis, are fuscous pigmentation and vacuolation. Fine vacuolation is not infrequent, but massive vacuolation of a striking character has occurred in some cells in cases 2-4 (fig. 9). Changes are also present in some of the cells of the lateral horns in all our cases except 3. Comparison with Gagel's (1928) description shows these changes to be pathological and chromatolytic in type. Satellitosis of nerve cells and glial nodules (Courcoux *et al.*, 1929; Courville and Mason, 1931) are not present, but there is a little increase in the number of astrocytic nuclei in the posterior horns in cases 3 and 5. Demyelination is not found, either irregularly or affecting tracts. In two cases longitudinal sections of the cord were cut.

Medulla. This was examined in cases 3 and 4. Chromatolysis

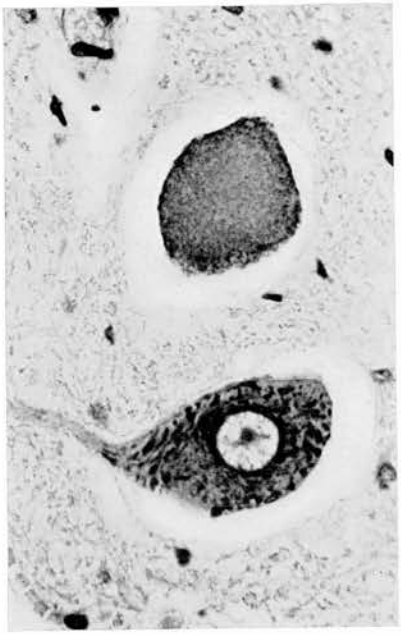

FIG. 8.—Case 3. Anterior-horn cells of thoracic cord; one is chromatolysed, its neighbour is undamaged. Cresyl-violet. ×500.

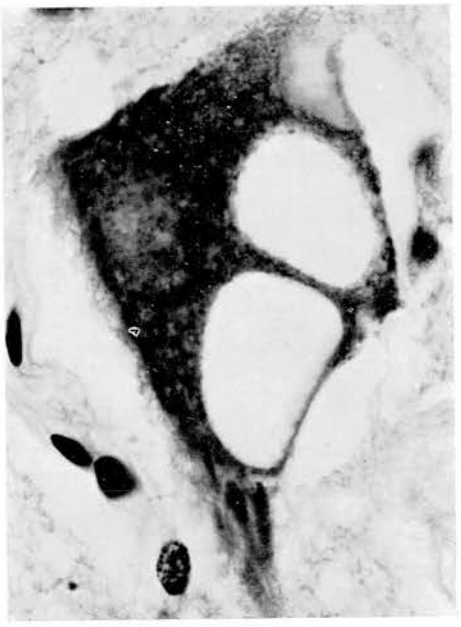

FIG. 9.—Case 4. Anterior-horn cell of lumbar cord, grossly vacuolated. Cresyl-violet. ×750.

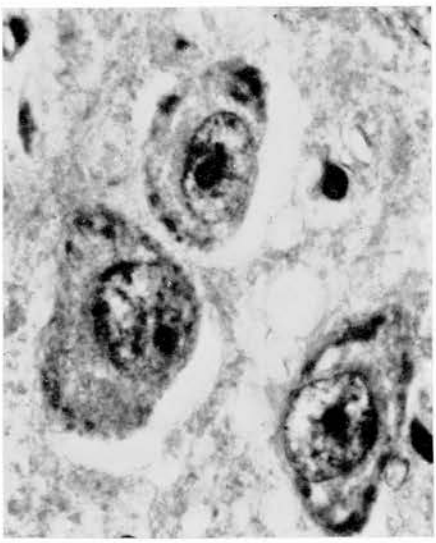

FIG. 10.—Case 4. Dorsal vagal nucleus; central chromatolysis. H. and E. ×750.

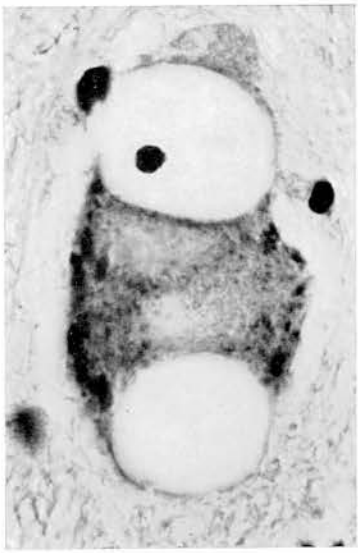

FIG. 11.—Case 3. Medulla. Nucleus ambiguus; a grossly vacuolated cell. Cresyl-violet. ×900.

(fig. 10) and massive vacuolation (fig. 11), similar to what is described in the cord, are seen in ganglion cells. No nucleus is completely spared. Chromatolysis is more advanced in the dorsal vagal nuclei than in the hypoglossal nuclei. Chromatolytic cells are also found in the nucleus solitarius and nucleus ambiguus and in the reticular substance.

Cerebellum. Foci of demyelination up to 4 mm. across are found in the white matter in Spielmeyer preparations in cases 1 and 5. They are similar in character to those described below in the cerebrum. In case 3 there is a little lipofuscin in macrophages around blood-vessels. In several cases the Purkinje cells are slightly shrunken.

Midbrain. There is striking chromatolysis of a group of oculomotor cells in case 5.

Cerebrum. Small foci of demyelination, generally 1-2 mm. across, are demonstrable in the 4 cases in which Spielmeyer's method was used. They are not found in paraffin sections stained by Loyez's method, a fact suggesting that the neurokeratin elements of the sheaths are relatively unaffected. The foci are frequently centred on blood-vessels (fig. 12), and sometimes preserve a central perivascular ring of myelin. At the edges of the demyelinated zones, globular fragments of sheaths are seen (fig. 13). In the larger foci there is partial loss of axons, which are fragmented and beaded (fig. 14), but this is less extensive than the myelin degeneration. As well as these clearly demarcated lesions, there are, in some instances, ill-defined areas of partial demyelination and also a little irregular intracortical demyelination (Abbott and Evans, 1946). The focal lesions are demonstrable more often in the parietal lobe than elsewhere, but they are also found in the frontal and occipital lobes. Although not present in every block, they are readily demonstrable in several blocks in each case. There is a slight and rather inconstant increase in glial nuclei in the demyelinated regions. The capillary endothelial cells are a little swollen. The cerebral lesions are also associated with small amounts of sudanophilic lipoid in perivascular macrophages. Elsewhere in the brain, yellow granules are seen in perivascular macrophages (fig. 15). This pigment, sudanophilic and periodic acid-Schiff-positive, is apparently the lipofuscin frequently present in this situation normally, but the amounts in our cases are a little excessive. In its distribution throughout the brain, the pigment is unrelated to the acute lesions. Similar pigment granules are found in the interstitial tissue of the choroid plexus in case 3 only. In this case and in case 5, the epithelial cells of the plexus contain a dust-like brown pigment. In addition to these focal changes, each brain is slightly œdematous (fig. 15). Chromatolysis is observed in some nerve cells of the cortex and basal nuclei, but it is less striking than in the cord and medulla. Many cells contain lipofuscin. Fine but not massive vacuolation is also seen. Changes occurring less regularly include pyknosis, ischæmic cell disease (Grogg, 1951 ; Schwarz and Moulton,

1954), indistinct staining (Mason *et al.*, 1933) and rarely satellitosis (Little and Palmer, 1948). Sommer's sector and the neighbouring portions of the hippocampus, examined in cases 3-5, are undamaged. Minor increases in glia are seen in a few areas of the hemispheres and in the basal nuclei not affected by demyelination. The type of enlargement and proliferation of protoplasmic astrocytes described by Adams and Foley (1953) in hepatic coma cases is not a feature of our porphyria cases.

Meninges. No lesions are present in any of the cases.

Fluorescence. Sections of the spinal cord of cases 3, 4 and 5, are shown by this method to contain fine granules with a reddish fluorescence in the white matter. These are somewhat less numerous than similar granules in the control sections. The granules resemble those described by Klüver (1944) as coproporphyrin. Golden fluorescence of lipofuscin is prominent in anterior-horn cells but no porphyrin fluorescence is seen.

Discussion

Peripheral and autonomic nerves

Scattered patchy demyelination of peripheral nerves was first described by Erbslöh (1903) in porphyria following sulphonal medication and subsequently by a majority of those who have examined fatal cases of acute porphyria. Mason *et al.* (1933) and Lapresle (1950) reported demyelination in the autonomic system. In the present cases breakdown or loss of myelin can be demonstrated by fat or myelin stains in the peripheral and autonomic nerves (figs. 2, 3 and 6). The observation by Mason *et al.* of perivascular lymphocytic infiltration was confirmed in the more severely affected nerves (fig. 5). The histological features do not clearly distinguish the nerve lesions of porphyria from several other neuropathies. The classical example of this type of lesion is the segmental periaxial neuritis of Gombault (1880-81) in experimental lead poisoning. Reference to the literature (Erbslöh, 1903 ; Courville and Mason, 1931 ; Lapresle, 1950) confirms that the myelin sheaths are the most severely damaged part of the conducting system in acute porphyria, and that this is where the system is primarily attacked and deprived of function. On recovery, conduction is doubtless restored by reconstitution of the myelin, most rapidly in those fibres in which the axon survives. Nevertheless some axons are destroyed in every badly damaged nerve (fig. 4), and if the attack is a severe one, or if frequent recurrences take place, so many axons with their myelin sheaths may be destroyed that clinical recovery is delayed many months or function is permanently impaired. Such advanced damage was seen in the nerve biopsy reported by Drury (1956) and in the brachial plexus in case 3 (fig. 6), which was grossly atrophied, though probably not beyond the possibility of clinical recovery. Hare and Wilmore (1948) and Adams

et al. (1953) described neural atrophy of muscle with persistent nerve lesions.

Any attempt to correlate precisely the histological changes in the peripheral nerves with individual clinical signs is of doubtful value in view of the patchy nature of the lesions on the one hand and the gradual onset and varying pattern of the paralyses on the other. Comparison with some other conditions is more instructive. The speed with which traumatic paralyses recover is largely dependent on the preservation or destruction of axon cylinders, and the terms neurapraxia and axonotmesis respectively are used by Seddon (1943) in describing such traumatic lesions in which the continuity of the nerve bundle is maintained. Much of the nerve damage in acute porphyria is comparable to neurapraxia and recovery of function is accordingly prompt. Individual nerves, however, as in trauma, often show a mixture of neurapraxia and axonotmesis and if the latter predominates recovery is considerably delayed. In the predominance of motor disturbances, the neuropathy of porphyria is also like traumatic lesions of minor severity, *e.g.*, tourniquet paralysis (Richards, 1954) and various forms of experimental nerve trauma (Denny-Brown and Brenner, 1944*a* and *b* ; Denny-Brown, Adams *et al.*, 1945). Motor fibres that are large and myelinated are the first to suffer in many types of lesion. Smaller sensory fibres are more resistant, and when affected in porphyria it is probably because they traverse zones of particularly intense damage in nerves.

A comparison with experimental nerve trauma helps us to date the lesions, although only approximately in view of the differences in size and composition of the nerves involved, the species of animal used and the more gradual onset of the neuropathy of porphyria. After light percussion (Denny-Brown and Brenner, 1944*b*) there is a strictly localised demyelination with preservation of most of the axis cylinders ; œdema and cellular reaction also occur and are maximal at 14 days. This is a close parallel to the less severe lesions in our cases. In experimental Wallerian degeneration, on the other hand, the peak period of cellularity is about 25 days (Abercrombie and Johnson, 1942 ; Noback and Montagna, 1952). This condition, however, does not closely resemble the neuropathy of porphyria, for it is characterised by universal axon damage and by the predominance of fat phagocytosis within Schwann sheaths. In our cases free macrophages were much commoner than Wallerian ovoids, and many axons escaped. Although Joseph (1947) has put forward evidence that the section of axons exerts less influence on the cellular reaction than does the amount of myelin damage, the dating of experimental Wallerian degeneration cannot be applied closely to our lesions. It is probable that such severe lesions as those in the phrenic nerves in case 4 (fig. 3) are little more than 2 weeks old. Gross neurological manifestations occurred in this case 2 weeks before death, and it is reasonable to suppose that it was shortly before this time that demyelination began. Less advanced nerve lesions were

found in cases 1, 2 and 5, in which the neurological histories were shorter.

In case 3 the cellular reaction at the sites of demyelination was less intense than in case 4. This may have been due to treatment with cortisone in case 3. The nerves were sclerotic on account of previous attacks, and it was difficult to compare the not inconsiderable degree of œdema (fig. 6) with that in more acute cases. Experimentally McColl and Weston (1953) found less cellularity and less collagen in nerves previously transected in cats to which cortisone was given than was the case in animals not receiving the drug, but œdema and the usual decrease in the concentration of myelin lipoids were not affected. It is therefore improbable that cortisone has any beneficial effect in limiting the paralyses by reducing œdema and it was ineffective as also was ACTH, in a case reported by Perrault *et al.* (1953).

Central nervous system

Small foci of demyelination are found in the brain, even in cases 1, 2 and 5 where the fatal attacks had been of short duration, and the peripheral nerves show only a minor degree of demyelination. Demyelination here is probably a fairly early event in an acute attack, although less severe than in the peripheral nerves. Cerebral demyelination has been described by a few other workers (Baker and Watson, 1945 ; Denny-Brown and Sciarra, 1945 ; Abbott and Evans, 1946). In our cases only a relatively small amount of sudanophilic lipoid had been freed and ingested by perivascular macrophages. Eichler's (1932) fatty degeneration of the capillaries was probably the same thing. In our cases the capillary endothelial nuclei are slightly swollen (Grogg, 1951), but we found none of the exudative perivascular changes described by Schwarz and Moulton (1954). Systematised tract degeneration is also absent and there is no clear evidence of it in any published case. In case 1, where the fatal illness was marked by nystagmus and other signs of cerebellar disease, demyelination is pronounced in the cerebellum. In the other cases, however, there is no association between the recorded symptoms and the site at which cerebral demyelination can be found. None of our cases had generalised convulsions, as described by Barker and Estes (1912) and Vannotti (1954).

The presence of brown pigment in various locations in the brain has been discussed by Eichler and by Mason *et al.* In our cases the pigment appears to be a lipofuscin not directly related to pyrrole metabolism. It shows no predilection for the acutely damaged areas, but there is a slightly excessive amount in several of our cases. It is most obvious in case 3, where it is present in the interstitial tissue of the choroid plexus as well as in perivascular macrophages (fig. 15) and nerve cells. In these it may have been deposited after demyelination in some of the numerous previous attacks in this 18-year-old

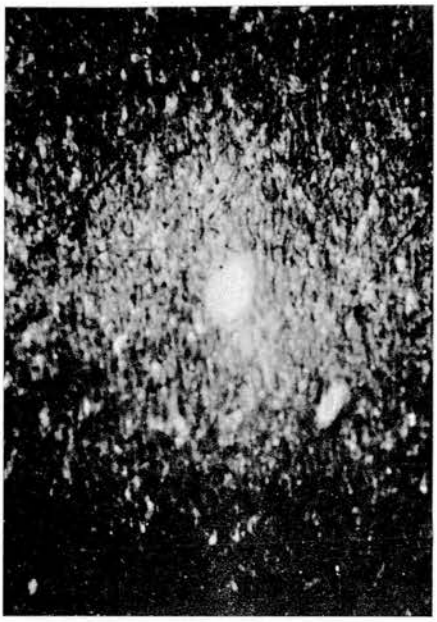

FIG. 12.—Case 3. Parietal white matter, showing a small perivascular focus of demyelination. Spielmeyer. ×30.

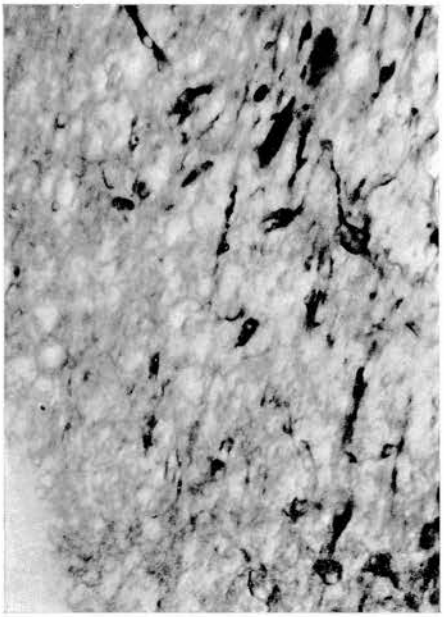

FIG. 13.—Case 3. Parietal white matter. The edge of a small perivascular focus of demyelination, showing degenerate and fragmented myelin sheaths. Spielmeyer. ×400.

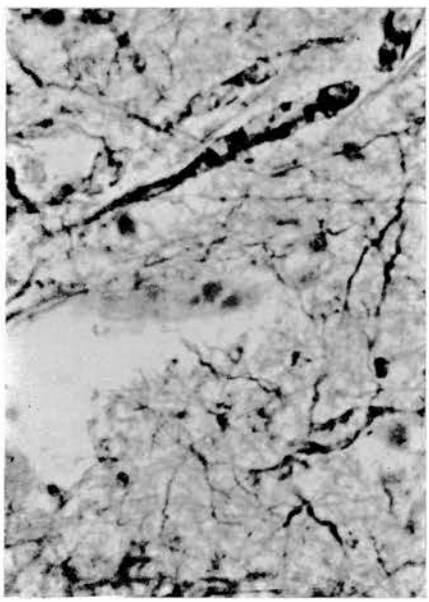

FIG. 14.—Case 2. Parietal white matter, showing fragmentation, irregular swelling and granularity of some axons crossing a demyelinated focus. Holmes's axon stain. ×540.

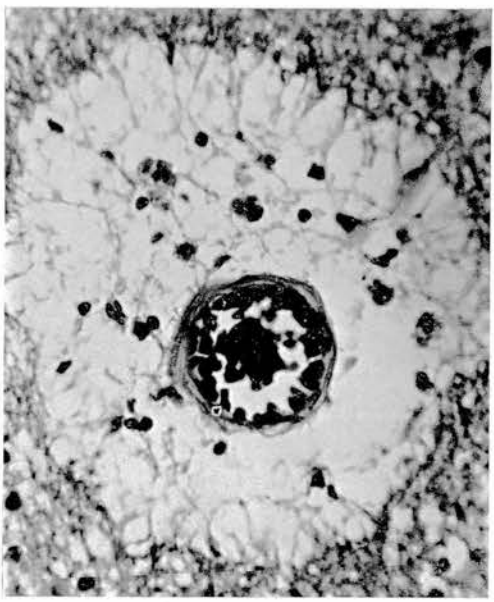

FIG. 15.—Case 3. Frontal lobe. An œdematous perivascular space with accumulated macrophages laden with lipofuscin. H. and E. ×350.

youth. In case 1, however, where a probable attack had taken place 9 years before, there was no increase of lipofuscin. Meningeal lesions have been reported by Golden (1943) and Schwarz and Moulton (1954), but they are exceptional and are not present in our cases. Vannotti reviews the reports of changes in the cerebrospinal fluid and notes that its examination generally gives negative results.

Chromatolysis of the anterior-horn cells of the spinal cord was first noted by Helweg (1892) in a case diagnosed as sulphonal poisoning, and later by Bostroem (1920) in acute porphyria. It is the commonest change in the central nervous system (fig. 8). Gray (1950) and Schwarz and Moulton (1954) noted chromatolysis in the splanchnic motor cells of the lateral horns of the cord. Chromatolysis is also common in the medullary nuclei (Denny-Brown and Sciarra ; Grogg ; Perrault *et al.* ; Schwarz and Moulton). In our cases it is most intense in the dorsal vagal nucleus (fig. 10). Although this is not always the case, the vagal nuclei are particularly mentioned by Mason *et al.*, by Lapresle and by Baker and Watson. There is chromatolysis in the oculomotor nucleus in case 5. Most of the changes in these sites are clearly retrograde or axonal in type (Denny-Brown and Sciarra ; Gray) and dependent on the lesions of the related nerve fibres and their sheaths. Probably many instances of chromatolysis in the cerebral cortex are also due to damage of the related nerve fibres through their being involved in demyelinated patches in the cerebral white matter, and we agree with Denny-Brown and Sciarra that the changes of most nerve cells, wherever they occur, are those of simple axonal reaction. These authors also drew attention to the presence of the vacuogranular change of Cajal in their case. In the cerebral neurones of our cases fine vacuolation (Eichler) is seen. Nerve cells of the spinal anterior horns and of the medulla, however, are massively vacuolated in several instances (figs. 9 and 11). Geissler (1939) and Courville (1945) also reported this in the anterior horns, and Courville pointed out that massive vacuolation is not specific for porphyria, since it occurs also, for instance, in amyotrophic lateral sclerosis. Nevertheless, it is sufficiently uncommon in other conditions and striking enough in itself to deserve particular note here. Other changes of the neurone cells and interstitial tissues of the brain, which we found inconstantly, must be considered non-specific and of little assistance in characterising the disease.

Pathogenesis

The occurrence of hypertension in some attacks of acute porphyria suggests that vasospasm may play a part in the pathogenesis of the lesions. Waldenström considers that retinal angiospasm is the cause of the amblyopia that sometimes takes place in the disease, and he emphasises the occasional coincidence of polyarteritis nodosa. His view deserves attention here, because there is some resemblance

between the peripheral-nerve lesions found in porphyria and in polyarteritis (Lovshin and Kernohan, 1948; Heathfield and Williams, 1954) and in ischæmia (Blackwood and Holmes, 1954). But this constitutes no evidence of a common cause, for they are in no way specific. Nor is there any satisfactory histological evidence that any of the cerebral damage in our cases was brought about by vasospasm. Sommer's sector and the neighbouring white matter are notably spared, therefore the long and vulnerable arteries of Ushimura had apparently not been affected. Furthermore, whether or not there had been hypertension could not be related to the presence or severity of the lesions. Vascular hypertonus is seen in some cases of acute porphyria, but there is no evidence that it results from the operation of a humoral agent, and Goldberg *et al.* (1954) were unable to demonstrate any action of porphobilinogen or of the naturally occurring human porphyrins on smooth muscle. It seems probable that hypertension is brought about by a nervous mechanism which we shall discuss. In a case of acute porphyria with polyarteritis, not referred to elsewhere in this paper, several uncomplicated attacks took place before the final one, which was accompanied by polyarteritis. It might be that demyelination of afferent pre-ganglionic vasomotor fibres in acute attacks of porphyria eventually makes the arteries more susceptible to polyarteritis.

The perivascular location of the cerebral demyelination in acute porphyria, shown in fig. 12, suggested to Baker (1950) the action of a blood-borne toxic agent. In our cases 3 and 4, in which the lesions generally were the most severe, the highest urinary uroporphyrin levels (77 mg. and 81·5 mg./l.) were recorded in the urines *post mortem*. Examination of fixed spinal-cord sections for porphyrin fluorescence, however, showed no abnormal concentration of porphyrins in the cells or tracts, a result that accords with the findings of Gray (1950). In cases of acute porphyria, Klüver (1951) and Schmid *et al.* (1954) found no increase of uroporphyrin in the brain and only a patchy and irregular amount in the peripheral nerves. Thus the evidence is entirely against the view that uroporphyrin is concentrated in any part of the nervous system in this disease. Further arguments against a direct toxic action of uroporphyrin are that in congenital porphyria large amounts of uroporphyrin are stored in the body without causing nervous symptoms, and that Goldberg *et al.* (1955), in experimental porphyria, found none of the physical manifestations of the natural disease except constipation and anorexia, although their animals excreted proportionately more uroporphyrin and porphobilinogen than do human patients. Porphobilinogen, it may be added, occurs in the urine of persons with latent porphyria who present no nervous manifestations. The fact that circulating porphobilinogen is rapidly excreted when renal function is normal (Goldberg, 1954) also makes it unlikely that porphobilinogen poisoning accounts for the nervous lesions.

Goldberg (1954) brought forward evidence that porphobilinogen is formed in the liver, and Watson and his school classify acute porphyria as porphyria hepatica. Schmid *et al.* state that some liver impairment is common in this group of porphyrias, and Vannotti found a positive galactose test in 30 per cent. of his cases. Baker reported demyelination rather similar to that in acute porphyria in 8 out of 18 cases of subacute and chronic liver disease, but Adams and Foley failed to confirm this. They described certain changes in the protoplasmic astrocytes in hepatic coma, but they did not find these changes in acute porphyria.

Clinical considerations

The histological changes in the nervous system in acute porphyria shed little light on the pathogenesis of the disease, but they are of considerable value in explaining the main clinical findings. Many of the symptoms are obviously related to the demyelinating lesions, *e.g.*, the phrenic-nerve lesions and respiratory paralysis in case 4. The predominance of motor damage has been discussed. The irregularly patchy involvement of the nervous system accounts for the notoriously varied manifestations of the disease. The diagnosis of acute porphyria cannot be made without the demonstration of porphobilinogen in the urine, but three clinical findings should suggest that a peripheral neuropathy is of porphyric origin, namely mental changes, abdominal pain and hypertension. These, though not unknown in other peripheral neuropathies, are common features of acute porphyria.

Mental changes were present in each of our cases, with the possible exception of case 5. Although the mental state may return to normal on physical recovery, psychosis may persist for many years after an attack, as in the niece of one of our patients (case 5) and in the case reported by Drury. It may be that an underlying mental instability is made manifest by an attack (Günther, 1922). Roth (1945) has suggested that porphyria occurs in families in which psychiatric disorders are rife, but it is also possible that the mental symptoms have some basis in structural damage. Spillane (1947) remarks that when cerebral demyelination occurs in pernicious anæmia it is usually found in patients who have had psychotic symptoms.

Pain is often the first indication of an attack, as in case 4. Some patients, like case 1, complain of pain in the limbs, which may be due to sensory-fibre damage. Posterior spinal-root-ganglion changes have been described by Bostroem, by Mason *et al.* and by Denny-Brown and Sciarra. It is abdominal pain, however, that is generally most severe and which characterises the attack. The abdominal pain is colicky and may be associated with vomiting, diarrhœa or constipation. Intestinal spasm alternating with atonia and dilatation and varying in location has occasionally been observed at laparotomy in these patients, and has been demonstrated both by intragastric balloon (Berg) and by radiography (Berlin and Cotton, 1950 ; Calvy

and Dundon, 1952). Vannotti reviewed the experimental evidence in favour of porphyrins, including the artificial substance hæmatoporphyrin, exerting a direct action on the intestine. He came to the conclusion that they have a specific effect on intestinal peristalsis and a secondary influence on the action of pharmacological substances affecting intestinal motility. Goldberg *et al.* (1954), however, failed to demonstrate any pharmacological action of the naturally occurring porphyrins or of porphobilinogen on the rabbit uterus and small bowel and on the guinea-pig ileum.

Consideration of the nerve lesions suggests another explanation of the pain. Pre-ganglionic motor fibres that innervate the viscera are myelinated and have their cell stations in the spinal cord and medulla. We have already referred to the retrograde degenerative changes found in these nuclei, and Berlin and Cotton suggest that such changes in the vagal nuclei might be important in relation to the abdominal pain. Chromatolysis in the nuclei reflects the demyelination of the dependent fibres, which was not extensive in any one place in our cases but was disseminated patchily along the nerves. Total interruption of conduction and paralysis of the bowel were therefore unlikely, and it is more probable that the motor innervation of affected segments had become irregular and, by thus disturbing the motility of the bowel, had led to abdominal pain. This view of the mechanism of the abdominal pain might explain the occasional relief which follows the administration of neostigmine or of ganglion-blocking agents (Berg, 1945 ; Gillhespy and Smith, 1954 ; Rimington and Goldberg, 1954). Günther (1922) recommended vagotomy for recurrent abdominal pain.

Hypertension is an important but by no means constant feature of attacks of acute porphyria. It may be transient during an attack, or it may be found in one attack and not in another in the same patient. An attack of porphyria may draw attention to an elevation of blood-pressure due to concurrent essential hypertension (our case 2 ; Saint *et al.*, 1954). Hypertension in acute porphyria is usually independent of respiratory embarrassment and cannot therefore be secondary to hypoxia as in some other conditions (Clarke *et al.*, 1954). It is possible, however, to explain its occurrence on the basis of lesions affecting the myelinated afferent fibres from the aortic and carotid sinuses in the ninth and tenth nerves. Kezdi (1954), who has investigated dysfunction of this system in acute porphyria, believes that demyelination interrupts the normal flow of inhibitory stimuli from these sinuses and leads to hypertension.

Summary

A histopathological study of the nervous sytem has been made in 5 fatal cases of acute porphyria, in each of which biochemical investigation had confirmed the diagnosis.

Demyelination of peripheral and autonomic nerves is prominent two weeks after the onset of paralysis. Axons are also damaged and Wallerian ovoids are seen, but demyelination occurs earlier and to a greater degree.

Small areas of perivascular demyelination are found in the white matter of the central nervous system. Retrograde degeneration of nerve cells is common, especially in the anterior horns of the spinal cord and in the medulla, where occasional cells show vacuolation.

Neuropathological changes are present whether or not the patient had been hypertensive, and there is no evidence that these have resulted from vasospasm.

The examination of sections for porphyrin fluorescence and our reading of the literature do not support the supposition that the nerve lesions result directly from a toxic action of porphobilinogen or of uroporphyrin.

The characteristic clinical findings of motor paralysis, abdominal pain, transient hypertension and mental changes are discussed in the light of the lesions demonstrated.

We wish to thank the many pathologists and physicians who generously made tissues and clinical histories available to us, and, in particular, Professor C. Rimington. We are grateful to Professors D. F. Cappell and J. H. Biggart for assistance in the preparation of the paper.

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PATHOLOGY OF ACUTE PORPHYRIA: EXPERIMENTAL PORPHYRIA

By A. Goldberg

Nuffield Unit for the Investigation of Pyrrole Pigment Metabolism, Department of Chemical Pathology, University College Hospital Medical School, London

In the past, the biochemical study of the porphyrin group of diseases in man and animals has added greatly to our knowledge of the pyrrole pigments. It is possible however that a more penetrating examination of the pathological changes in these diseases, natural and artificially produced, might contribute significantly to our understanding of the biochemical mechanisms involved.

The most important of the porphyria group of diseases—and also the most distressing—is acute intermittent porphyria, characterized by the excretion of large amounts of porphobilinogen in the urine. This disease occurs predominantly in young adults, especially women, who suffer severe attacks of abdominal pain, are very constipated, and sometimes have an elevation of blood pressure. Not infrequently paralysis of the limbs and, even more serious, of the diaphragm supervene. Severe mental disturbances and personality changes are not uncommon. The frequency of paralysis and mental change suggests some relationship between the metabolism of the nervous system and the disordered pigment metabolism.

Many of these patients die, and the remarkable thing about the pathological histology of autopsy tissues is that they often appear normal. Nevertheless, in some cases, definite microscopic changes have been noted, particularly in the liver, kidney and nervous system. In the liver, fatty degeneration, cellular necrosis and even cirrhosis (Vannotti, 1954) have been described. In the kidney, inflammatory lesions of the renal arteries and degenerative lesions of the convoluted tubules have been noted. Prunty (1949) has suggested that these tubular changes might be responsible for the electrolyte abnormalities sometimes observed in acute porphyria. Infection of the renal tract may precipitate an attack of the disease. As long ago as 1903 Erbslöh pointed out that myelin degeneration occurred in the peripheral nerves of a patient with porphyria following sulphonal intoxication. This finding has been confirmed several times in isolated cases, particularly by Mason, Courville & Ziskind (1933) and by Denny-Brown and Sciarra (1945). Dr J. B. Gibson of the Western Infirmary, Glasgow, after a careful study of four post-mortem cases, has been able to show clearly that demyelination occurred in every case in the peripheral nerves or in the central nervous system.

The opportunity has occurred of analysing chemically the tissues of six fatal cases of acute porphyria. The results of these investigations have amply confirmed the findings of Prunty (1945) and Gray (1950) that the liver and kidney contain porphobilinogen. Porphobilinogen has also been found in the plasma. There is now a general agreement that the liver is probably the seat of the pigment disturbance in this disease.

Clearly there are great limitations to the amount of investigation one can carry out on a human case of porphyria. There was therefore much importance in the report of Schmid & Schwartz (1952) that they had produced an experimental porphyria in rabbits using Sedormid (allyl-*iso*propyl-acetylurea) with the excretion of large amounts of porphobilinogen. They suggested that the experimental porphyria in these rabbits bore a resemblance to the clinical picture of the human disease. In particular they stated that when the urinary uroporphyrin reached levels of 7 mg./day transient paralysis, especially of the hind legs and bladder, was observed. We have confirmed the chemical findings of these authors, but have found that Sedormid, which is a profound hypnotic, obscures the clinical assessment of the animals. This has led us to investigate related drugs, one of which might produce the same *chemical* features, but yet be *non-hypnotic*. Such a drug is allyl-*iso*propyl-acetamide (Goldberg, 1953).

We have studied the effect of this drug in rabbits, fowls and rats and have concluded that, although the ' chemical porphyria ' produced in these animals is similar to the human disease, there are several important differences (Goldberg & Rimington, 1954).

Sedormid causes a rise of urinary coproporphyrin and of faecal coproporphyrin and of protoporphyrin almost as soon as the drug is given and, after about eight days, the excretion of uroporphyrin and porphobilinogen. These effects cease when the drug is discontinued.

Allyl-*iso*propyl-acetamide has a similar chemical effect (Fig. 1) but, since the drug is non-hypnotic, the animals have been observed for longer periods than with Sedormid, in one case for three weeks. The rabbits have no haematological changes (suggesting an ' over-production ' rather than an ' under-utilization ' (Drabkin, 1951) of haem precursors), show no evidence of hypertension and do not develop paralysis. They lose appetite, weight and are constipated. They may recover to a normal excretion of porphyrins if the drug is stopped, or they may die during the course of the drug from bronchopneumonia. One rabbit died in severe uraemia, and histology of the kidney showed evidence of renal tubular damage.

Experiments were carried out on 15 rabbits using Sedormid or allyl-*iso*propyl-acetamide. In reviewing the results it was found that 10 out of the 15 fell into two groups, the remaining five could not be so

clearly classified. Of these 10, six produced large amounts of porphobilinogen and were therefore called ' good excretors ' (Table 1). The livers of all these animals remained histologically normal and contained porphobilinogen. The only change in urinary amino-acid excretion was a greatly diminished amount of glycine.

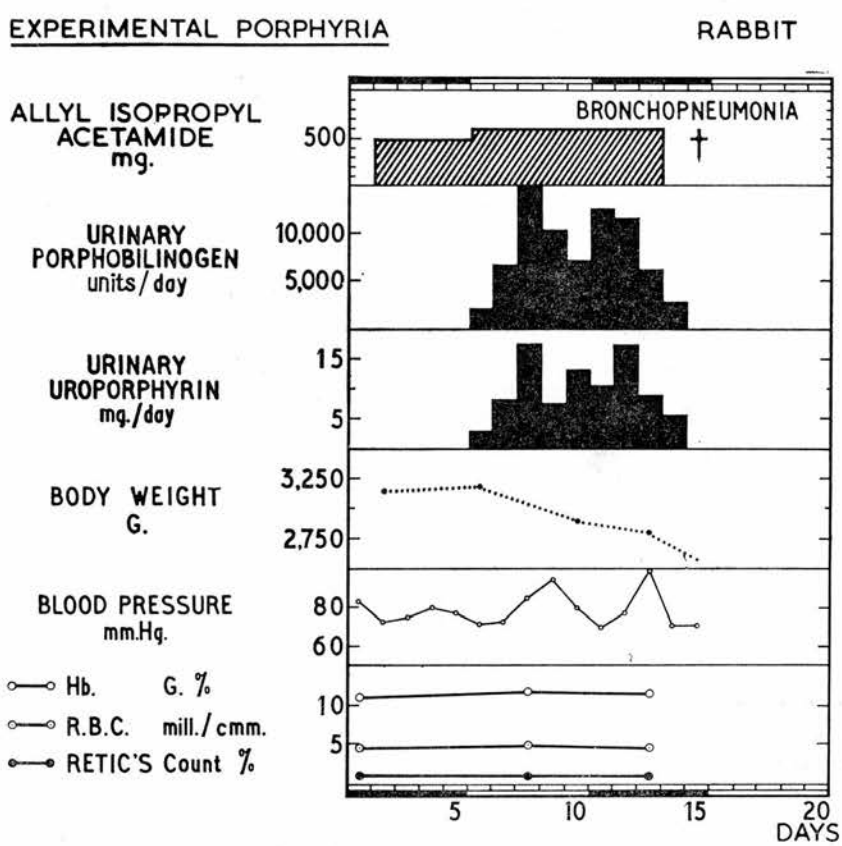


Fig. 1. Urinary porphobilinogen (for units see Westall, 1952) and uroporphyrin excretion, body weight, blood pressure, and blood picture of a rabbit treated with allyl-*iso*propyl-acetamide. The urinary uroporphyrin refers to that determined spectrophotometrically after heating urine with 2N-acetate buffer pH 4·2 for 20 minutes to convert porphobilinogen present to uroporphyrin.

On the other hand, four rabbits, on the same dose of these drugs, produced only small amounts of porphobilinogen, and eventually died with severe hepatic damage and an abnormal urinary amino-acid pattern (see Plate). The livers of these animals contained no porphobilinogen. The comparison of these two groups emphasizes the role of the liver in the production of porphobilinogen.

These studies (with allyl-*iso*propyl-acetamide) have been extended to fowls and rats. Fowls were used since these animals are known to

Table 1. *Comparison of ' good ' and ' poor ' excretors of porphobilinogen among* 10 *rabbits treated with allyl-*iso*propyl-acetamide or Sedormid*

Mean uroporphyrin excretion refers to the urinary uroporphyrin determined spectrophotometrically after heating urine with 2N-acetate buffer pH 4·2 for 20 minutes to convert porphobilinogen present to uroporphyrin.

Rabbit No.	Mean uroporphyrin excretion (mg./day)	Final urinary amino-acids	Liver Porphobilinogen	Histology
4	10·21	Glycine diminished	+++	Minor peri-portal fatty change
5	6·32	Glycine diminished	Animal still on drug after 23 days	
6	11·62	Glycine diminished	+++	Some fatty change. No necrosis
7	10·26	Glycine diminished	0*	Parenchyma normal
11	6·6	Not determined	+++	Parenchyma normal
12	11·29	Glycine diminished	+++	Parenchyma normal
1	1·52	Many present	0	Cellular necrosis
2	0·43	Not determined	0	Mid-zone degenerative changes
3	1·47	Many present	0	Cellular necrosis
10	1·53	Many present	0	Cellular necrosis

* No drug given during last two days of life. Died from bronchopneumonia.

be susceptible to metabolic derangement of their nervous system. In none of these animals—rabbits, fowls or rats—have frank paralyses been observed. Dr Fenton, of University College Hospital Medical School, has carried out histopathological studies on the nervous system of the rabbits and fowls used in these experiments and has failed to find any direct evidence of myelin degeneration.

The results of this work suggest that, in the animals used, the artificial production of a pigment dyscrasia, apparently the same as in human acute porphyria, is not associated with the same clinical picture. In the past it has frequently been inferred that the porphyrin pigments and porphobilinogen were the direct cause of the clinical signs and symptoms of the human disease. Recent work (Goldberg, Paton & Thompson, 1953) has shown that the porphyrins and porphobilinogen appear to be pharmacologically inactive. All this might suggest that the clinical picture in the human disease is brought about through a more subtle mechanism than was originally anticipated.

Since the introduction of barbiturates, in 1903, there have been conflicting reports on the possible relationship of these drugs to human

acute porphyria. A survey has therefore been made of the effect of nine different barbiturates on the porphyrin metabolism of rabbits (Goldberg, 1954). The results show that these barbiturates may be classified into four groups:—

1. Diallyl barbituric acid, which caused a considerable increase in the urinary coproporphyrin III output of eight rabbits, and in three of these also the production of porphobilinogen and uroporphyrin III.

2. Allyl-*iso*propyl barbituric acid and sodium allyl (1-methylbutyl) barbiturate, both containing one allyl group, which caused a moderate increase of coproporphyrin III.

The remaining two groups have a slight effect or no effect on porphyrin excretion (Fig. 2).

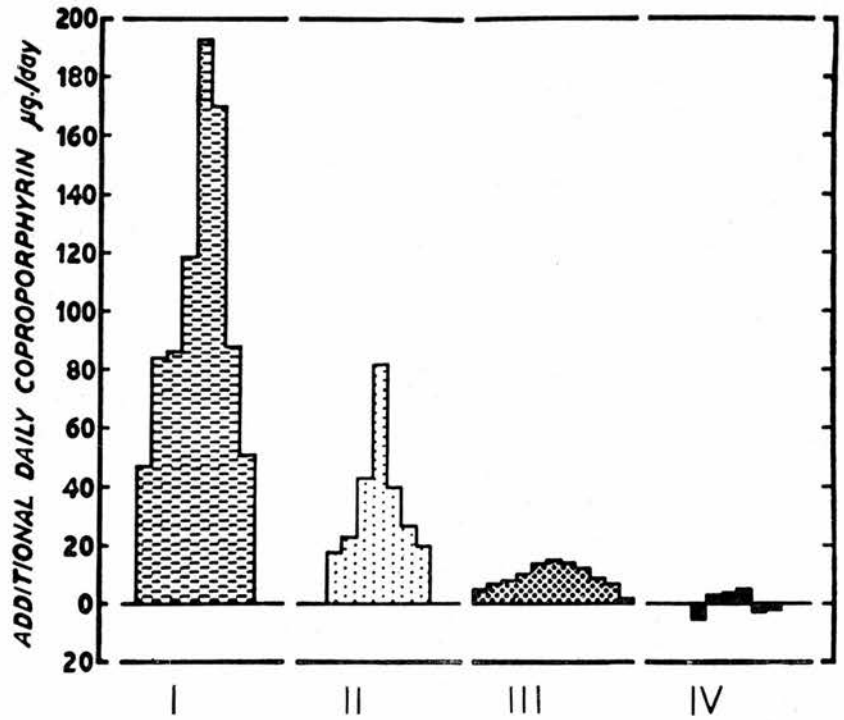


Fig. 2. *Effect of barbiturates on urinary excretion of coproporphyrin in rabbits.* Each vertical segment represents the mean daily coproporphyrin output (μg./day) in a single rabbit during barbiturate administration in excess of, or less than, the mean daily coproporphyrin excretion before and after the drug was given.

I. 5 : 5-diallyl barbituric acid.

II. Sodium 5-allyl 5-(1′-methylbutyl) barbiturate. 5-allyl-5-*iso*propyl barbituric acid.

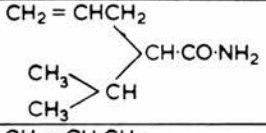
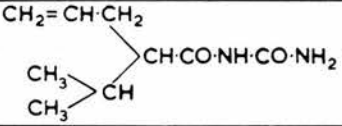
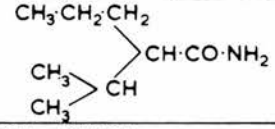
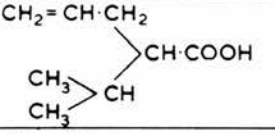
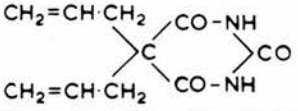
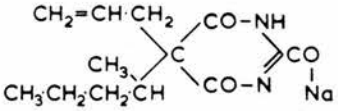
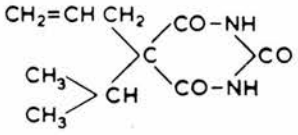
III. Sodium 5 : 5-diethyl barbiturate. Sodium 5-ethyl 5-(1′-methylbutyl) barbiturate. Sodium 5-ethyl 5-phenyl barbiturate.

IV. Sodium 5-*iso*-amyl 5-ethyl barbiturate. Sodium 5-butyl 5-ethyl barbiturate. Sodium 5-ethyl 5-(1′-methylbutyl) thiobarbiturate.

Tissue porphyrin determinations in rabbits, sacrificed at the height of porphyrin excretion, showed the liver and bile to be the only tissues with increased porphyrins.

On the basis of all these drug experiments there has been defined a chemical structure, which may interfere with normal porphyrin formation (Table 2). From this it has been concluded that the structure is at least one allyl group, together with an acid amide, ureide or a cyclic ureide, as in the barbiturate series.

Table 2. *Chemical structure and effects of certain drugs on the porphyrin metabolism of rabbits*

Drug	Structure	Effect on urinary porphyrin excretion
Allyl-isopropyl-acetamide (A.I.A.)		+++
Allyl-isopropyl-acetyl-urea. (Sedormid)		+++
n-Propyl-isopropyl-acetamide.		—
Allyl-isopropyl-acetic acid.		±
Diallyl-barbituric acid. (Dial)		++
Sodium allyl (1-methyl butyl)-barbiturate (Seconal)		+
Allyl-isopropyl-barbituric acid.		+

+++ Very marked effect (much coproporphyrin, porphobilinogen and uroporphyrin).
++ Marked effect (much coproporphyrin; some porphobilinogen and uroporphyrin in three out of eight rabbits).
\+ Moderate effect (coproporphyrin only).
± Slight rise in coproporphyrin only.
— No effect.

Just how this work may help in the elucidation of the problems raised by the human disease is a matter for conjecture. Clearly important points are (*a*) the nature of the hepatic mechanism deranged in experimental porphyria and its relation to that in human acute porphyria, and (*b*), perhaps of greater significance to clinical treatment, what is the association of the nervous system metabolism and pyrrole pigment metabolism, an association which is suggested by the pathological findings in the human disease.

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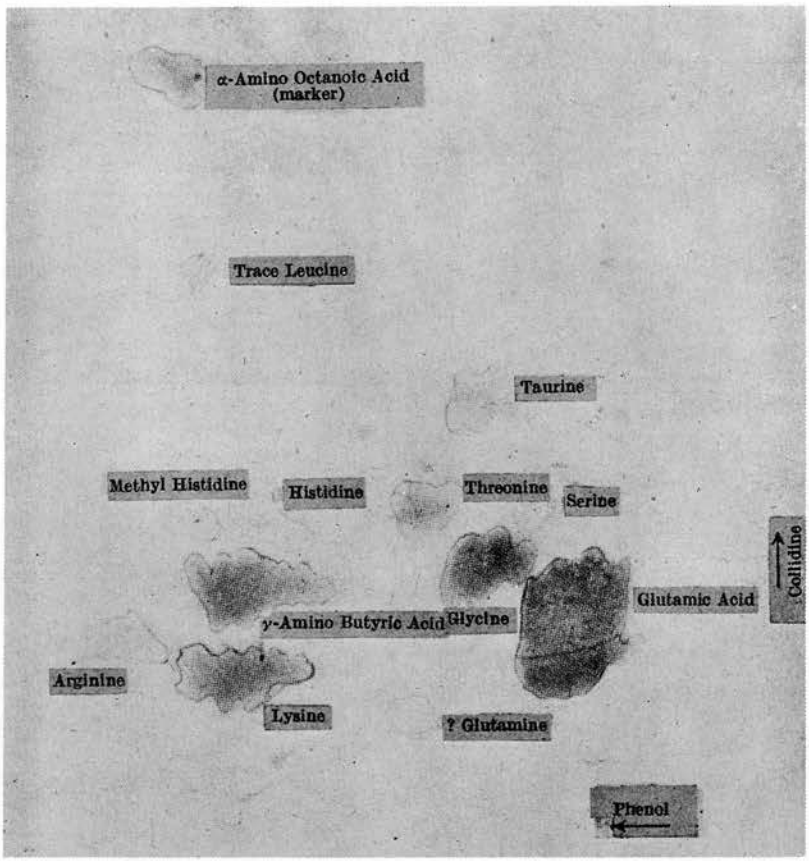
Plate



Amino-acid chromatogram (Dent, 1948) of the urine of a 'poor' excretor rabbit at the end of a course of Sedormid. An equivalent volume of urine from this rabbit before the drug was given showed only a solitary glycine spot on a paper chromatogram.

PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

BY

A. GOLDBERG, W. D. M. PATON, and J. W. THOMPSON

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PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

BY

A. GOLDBERG,* W. D. M. PATON, AND J. W. THOMPSON

From the Departments of Chemical Pathology and Applied Pharmacology, University College Hospital Medical School, London, W.C.1

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This paper attempts to define the relationship between the abnormal substances excreted in acute porphyria and the clinical manifestations of this disorder of porphyrin metabolism. During an attack of acute porphyria, patients usually excrete large quantities of porphobilinogen either alone or with certain porphyrins. The excretion of the porphyrins and porphobilinogen is usually in direct proportion to the severity of the symptoms, suggesting a causal relation, although Waldenström (1939) has reported an authenticated case in which the patient did not pass uroporphyrin or porphobilinogen in the urine or bile during the attack, but did so on other occasions. Several authors have claimed that porphyrins may influence the intestine or uterus (Supniewski, 1927; Gunther, 1922; Reitlinger and Klee, 1928; Vannotti, 1937; and Simici, 1938). Critical appraisal of these reports has led us to repeat this work using porphyrins of the kind known to be excreted in porphyria, which were obtained by improved methods of purification. The isolation of porphobilinogen in crystalline form (Westall, 1952) has for the first time allowed pharmacological testing of the pure substance, although Waldenström and Wendt (1939) and Prunty (1945) had injected partially purified porphobilinogen into rabbits.

METHODS

Animal Experiments.—Observations were made on the blood pressure (recorded with a cannula in the carotid artery) and on the respiration of 13 cats and 3 rabbits (anaesthetized with chloralose (80 mg./kg.) after induction with ether) and of 1 pithed cat, 1 pithed and eviscerate cat, and 1 decerebrate cat. Injections were made into the right femoral vein or the splenic vein.

Stimulation of the distal end of the vagus, separated and cut in the neck, was with supramaximal 0.5 msec. shocks at 10 c./s.

Isolated Organs.—Experiments were also made on isolated strips of guinea-pig ileum, non-pregnant rabbit uterus, rabbit jejunum and ileum, or cat ileum, set up in Tyrode's solution at 34° C. Contractions were recorded on smoked paper by a frontal writing lever. Experiments with light irradiation were done with an electric bulb of 300 w. at 25 cm. from the tissue in the organ bath.

Drugs.—The porphyrins, with the exception of haematoporphyrin, had been isolated from biological material as the methyl esters. Before use the esters were hydrolysed with 7 N HCl for 36 hours, at room temperature, the excess of HCl being then removed in a vacuum desiccator over KOH. Haematoporphyrin was prepared and used as the dihydrochloride. Pure crystalline porphobilinogen (Westall, 1952) was used; the porphobilin was obtained by Mr. R. G. Westall as a by-product in the preparation of porphobilinogen.

Porphobilinogen was dissolved in a minimum volume of 0.1 N $NH_4.OH$ and then made up to the required volume with 0.9% saline. For the porphyrins and porphobilin M/7 sodium bicarbonate was used as the solvent.

RESULTS

Anaesthetized Cats and Rabbits.—Recordings were made of the direct effect of porphobilinogen—and, in one experiment, of uroporphyrin I—on the blood pressure, respiration and vascular responses of the treated animals to acetylcholine, histamine, nicotine, adrenaline, noradrenaline, and vagal stimulation. The amount of porphobilinogen injected (up to 100 μg./kg.) was limited by the amount available; but from the known rate of excretion in patients (40–160 mg./day), and from the fact that tests for plasma porphobilinogen sensitive to 1 μg./ml. may be negative even at the height of an attack, it is likely that the blood levels obtained in our experiments were comparable to or even greater than those obtaining in acute porphyria. The only effect observed was an apparent potentiation by porphobilinogen of the response to adrenaline and noradrenaline in a few of the early

* Member of Nuffield Unit for the investigation of Pyrrol Pigment Metabolism, Department of Chemical Pathology.

experiments (Fig. 1). This apparent potentiation of adrenaline and noradrenaline could not, however, be repeated and its interpretation is complicated by the fact that considerable spontaneous fluctuations in sensitivity to these drugs may occur.

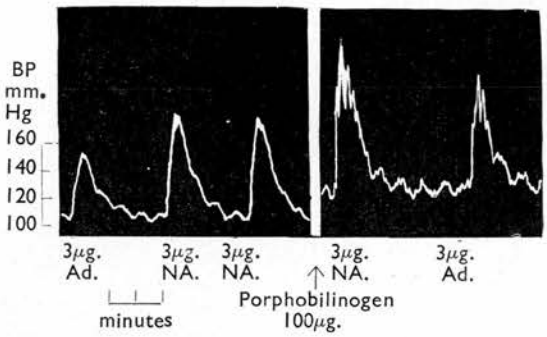

FIG. 1.—Cat.; chloralose; blood pressure recording; intravenous injections. Responses to 3 μg. adrenaline and noradrenaline before and after 100 μg. porphobilinogen.

Isolated Organs.—After obtaining records of spontaneous activity and tone, and of consistent responses to acetylcholine, histamine, adrenaline, and 5-hydroxytryptamine, the effect of adding porphyrins, porphobilinogen, and porphobilin to the preparation was investigated. With uroporphyrin I the effect of light irradiation was also determined. A summary of these results is given in Table I.

The only significant responses were those to haematoporphyrin (1/8,000) and to porphobilin. The former produced a distinct waning contraction of guinea-pig ileum, followed by inactivity of the intestine and a refractoriness—which became complete—to histamine and acetylcholine. Rabbit intestine was unaffected. Porphobilin produced a histamine-like contraction, sensitive to mepyramine, but less so to atropine (Fig. 2); it was considered likely that the effect was due to contamination (c. 0.4 mg./g.) with histamine itself.

Test of Porphobilinogen on Unanaesthetized Rabbit.—10 mg. porphobilinogen was injected intravenously into a rabbit (2.2 kg.) with an external biliary fistula. The animal showed no abnormal symptoms in the 3 days following the injection. There was a slight rise in the level of bile protoporphyrin during this period and a trace of uroporphyrin was noted in the urine several hours after the injection. No porphobilinogen was found in the urine.

Test of Whole Urine from Patients with Acute Porphyria.—As a final test, to cover the possibility that in porphyria some unidentified pharmacolo-

TABLE I

TESTS OF PORPHOBILINOGEN AND PORPHYRINS ON ISOLATED TISSUES

		Porphobilinogen	Uroporphyrin I	Uroporphyrin III	Coproporphyrin I	Coproporphyrin III	Haematoporphyrin	Porphobilin
Rabbit uterus	Drug concn.	1/40,000	1/10,000	1/40,000	1/40,000	1/40,000		
	Effect on spontaneous activity and tone	0	0	0	0	0		
	Effect on response to adrenaline	0	0	0	0	0		
Rabbit ileum or jejunum	Drug concn.	1/40,000	1/10,000				1/10,000	
	Effect on spontaneous activity and tone	0	0				0	
	Effect of combining with light irradiation		0					
	Effect on response to histamine, acetylcholine, adrenaline						0	
Guinea-pig ileum	Drug concn.	1/20,000	1/40–10,000	1/10,000	1/20,000	1/20,000	1/8,000	1/20,000
	Effect on spontaneous activity and tone	0	Slight + twice only	0	+ twice only	0	–	+ (histamine contaminant ?)
	Effect on response to acetylcholine	0					–	
	Effect on response to 5-OH tryptamine	0		0	0	0		
	Effect on response to histamine		+ (50%) once only				–	

0 = No effect: + = increase; – = decrease.

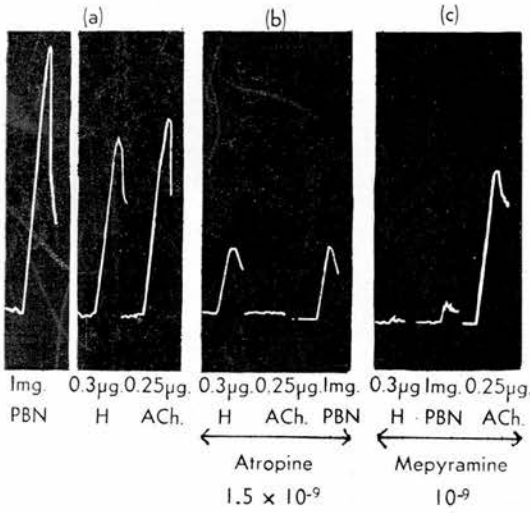

FIG. 2.—Isolated guinea-pig ileum. Response to 1 mg. porphobilin, 0.3 μg. histamine and 0.25 μg. acetylcholine (a) normally, (b) in presence of 1.5×10^{-9} atropine, (c) in presence of 10^{-9} mepyramine.

gically active substance is excreted, a sterile specimen of urine from a patient suffering a moderately severe attack of acute porphyria (with hypertension and abdominal pain) was infused into an anaesthetized cat at a rate of approximately 4 ml./min. for 15 min. This by itself produced no effect on blood pressure or respiration; and its effect on the responses to adrenaline, noradrenaline, histamine, acetylcholine, and nicotine was indistinguishable from that of a specimen of normal urine.

DISCUSSION

Despite a great deal of research on acute porphyria the mechanism of the production of symptoms remains obscure. Recent work has tended to minimize the possible direct influence of the porphyrins (Waldenström, 1939) and to emphasize the importance of the pathological features of patchy myelin change observed in the peripheral and autonomic nerves (Denny-Brown and Sciarra, 1945). These authors considered that the changes might be caused by an intermittent ischaemia, probably due to a circulating vasoconstrictor substance. Following this, Wehrmacher (1952) reported clinical improvement in acute porphyria with the use of ganglion-blocking agents. A search for some such vasoconstrictor substance, which might be present only in active cases of acute porphyria, would be a reasonable approach to the problem. The pharmacological testing of the known and already purified excretion products was clearly necessary. It would be unlikely that uroporphyrin and coproporphyrin could fulfil this role, since these are excreted in increased amounts in both congenital porphyria (as the series I isomers), and in porphyria cutanea tarda (as the series I and III isomers) where skin photosensitivity may be the only symptom. Porphobilinogen, however, is always excreted in the urine in attacks of acute porphyria and in those phases of porphyria cutanea tarda where acute symptoms are superimposed on the cutaneous syndrome. For this reason porphobilinogen or some closely related substance has been strongly suspected of being the *materia peccans* of acute porphyria (Lowry *et al.*, 1950).

Our experiments have failed to show that either the porphyrins or porphobilinogen have any significant pharmacological action. The initial animal experiments, in which porphobilinogen appeared to potentiate the blood pressure responses of the cat to adrenaline and noradrenaline, could not be repeated. These interesting results cannot be explained, although it is just possible that there may be an individual tissue and animal sensitivity to such drugs. Their inactivity in our hands is at variance with the results of some previous investigators. The difficulty of isolating porphyrins from biological materials, such as urine, in a state of purity that will guarantee freedom from possible histamine contamination, is very great. Our experience suggests that the contradictory results obtained by previous workers may possibly have been due to histamine contamination.

The results of intravenous injection of porphobilinogen into a rabbit confirm Prunty's (1945) findings, but contradict those of Waldenström and Wendt (1939), who found porphobilinogen in the urine of a rabbit into which they had previously injected the partially purified substance (amount used unknown).

Further work has tended to substantiate the absence of pharmacological activity of the porphyrins and porphobilinogen. An "experimental porphyria" or disturbance of porphyrin metabolism has been produced in rabbits by the non-hypnotic substance allyl *iso*propyl acetamide (Goldberg, 1953); very large quantities of uroporphyrin and porphobilinogen were excreted—in one animal for as long as three weeks—without obvious pharmacological effect. Apart from constipation, there is no evidence that the state of these rabbits compared with the clinical state of human acute porphyria, although the animals excreted proportionately greater quantities of the substances. Falk, Dresel, and Rimington (1953) have shown that porphobilinogen is a porphyrin precursor in a tissue system. This emphasizes that in porphobilinogen

we are probably dealing with a physiological substance. While our experiments therefore do not rule out the possibility that an unidentified vasoconstrictor substance may be produced in acute porphyria, or that the known excretion products may exert a pressor action by a mechanism at present unknown, they render these suggestions unlikely.

SUMMARY

1. The porphobilinogen and the porphyrins usually excreted in acute porphyria, as well as haematoporphyrin and porphobilin, have been tested pharmacologically.

2. Apart from slight and variable action of uroporphyrin I, coproporphyrin I, and porphobilin, these substances show no pharmacological action.

3. The significance of the results in relation to the symptoms of acute porphyria is discussed.

We wish to thank Mr. R. G. Westall for generous supplies of porphobilinogen and Professor C. Rimington for specimens of porphyrins, and constant help through these experiments.

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RENAL CLEARANCE OF ENDOGENOUS PORPHOBILINOGEN IN MAN

A. GOLDBERG
M.B. Edin., M.R.C.P., F.R.F.P.S.

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A. GOLDBERG
M.B. Edin., M.R.C.P., F.R.F.P.S.
NUFFIELD RESEARCH FELLOW, DEPARTMENT OF CHEMICAL PATHOLOGY, UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL, LONDON

EVIDENCE put forward recently suggests that porphobilinogen injected parenterally in the rat is cleared from the blood at a rate similar to the expected glomerular filtration-rate (Goldberg and Rimington 1954, Goldberg 1954). I report here studies on the renal clearance of endogenous porphobilinogen in three cases of acute intermittent porphyria (two during an acute attack and one during a remission) and in one case of latent porphyria. The results suggest that in these patients also endogenous porphobilinogen was excreted by glomerular filtration with little or no tubular reabsorption.

Methods and Materials

CLINICAL

The Cases

Case 1.—Mrs. A., aged 23. Height 155 cm., weight 43·6 kg. Acute intermittent porphyria. This was her first attack. She had had recurrent bouts of abdominal pain, transient hypertension, and tachycardia for 6 weeks. There was weakness of her arms and legs. The experiment was done during the acute phase on a day when she was coöperative and had little abdominal pain. She excreted 215 mg. of porphobilinogen in her urine on that day.

Case 2.—Mrs. B., aged 23. Height 162 cm., weight 55·5 kg. Acute intermittent porphyria. This was her first attack. She had had severe abdominal pain and vomiting for 3 weeks and had difficulty in passing urine. On the day of the experiment her symptoms had greatly improved and she excreted 52 mg. of porphobilinogen in her urine.

Case 3.—Mr. C., aged 29. Height 193 cm., weight 74 kg. Latent porphyria. Situs inversus totalis. Bronchiectasis. Traumatic epilepsy. He has never had symptoms suggesting acute intermittent porphyria, yet he excretes about 100 mg. of porphobilinogen in his urine each day. (Patient of Dr. Donald Hunter, London Hospital.)

Case 4.—Mr. D., aged 26. Height 175 cm., weight 65·5 kg. Acute intermittent porphyria in remission. A year ago he

had a very severe attack with quadriplegia. He still has wasting of the small muscles of his hands. He excretes about 120 mg. of porphobilinogen in his urine daily. (Patient of Dr. E. Frankel, Wanstead Hospital, London.) Two separate experiments were made in this case: (*a*) clearance of endogenous porphobilinogen, and (*b*) simultaneous clearance of endogenous porphobilinogen and of inulin.

Cases 1 and 2 were patients in University College Hospital; and cases 3 and 4 were outpatients and had been admitted to the metabolic ward of University College Hospital the night before the experiment. All the patients were recumbent in bed throughout each experiment, except case 3, in whom the effect of postural change on the renal clearance of porphobilinogen, creatinine, and urea was investigated.

Food and Fluids

Each patient was given breakfast before the start of the experiment. Cases 1 and 4(*a*) in addition had a light lunch during the experiments. Cases 2, 3, and 4(*b*) fasted throughout the experiments. The patients were encouraged to drink water and orange-juice from an hour before till the end of the clearance periods.

Collection of Urine and Plasma

Urine was passed voluntarily by all the patients except case 2, who had to be catheterised at the end of the clearance period. The minimum of urine passed in any single clearance period was 230 ml. The mean of all such volumes was 544 ± 260 ml.

The experiments in every case were made between 7 A.M. and 5 P.M. In case 1 the urine excreted in 6 hours (from 11 A.M. till 5 P.M.) was collected, and plasma specimens were taken at 1, 3, and 5 hours after the start of the experiment. Case 2 emptied her bladder at 7 A.M. and was catheterised $4^1/_4$ hours later. Plasma specimens were taken at 1 and 3 hours after the start of the experiment. In cases 3 and 4(*a*) the patients had four and five consecutive 2-hour clearance periods beginning at 7 A.M. and 9 A.M. respectively. Plasma specimens were taken an hour after the start of each clearance period. In case 4(*b*) the clearance of endogenous porphobilinogen was studied for 2 hours (from noon to 2 P.M.). An intravenous inulin drip had been set up at 11 A.M., and inulin clearance was determined for the period 12.30–2.0 P.M. The inulin clearance recorded represents the average of three separate half-hourly inulin clearances recorded in this $1^1/_2$ hours. The minimum of urine passed during each half-hourly period was 194 ml., and the mean volume for the three periods was 240 ± 35 ml.

Urea and creatinine clearances were measured in addition to porphobilinogen clearances in certain cases (see table).

BIOCHEMICAL DETERMINATIONS

Porphobilinogen was determined in urine and plasma by methods described by Goldberg (1954). In every case 1·5 ml. of 20% (*w*/*v*) trichloracetic acid was added to 6 ml. of plasma.

Weighed quantities of crystalline porphobilinogen were added to distilled water and normal human plasma, sufficient to give concentrations in the same range as that of the specimens of urine and plasma to be tested. The densities of these standard aqueous and plasma solutions when treated with Ehrlich's reagent were measured at 552 mμ on a 'Unicam' spectrophotometer (S.P.500), and calibration curves were drawn, the spectrophotometric readings being plotted against known concentrations of porphobilinogen in water and plasma.

The urine in each case was diluted 25–100 times with distilled water. It was therefore considered permissible to measure the porphobilinogen content of this very diluted urine by the calibration curve of the standard aqueous porphobilinogen solutions. The porphobilinogen contents of patients' plasma were likewise calculated from the calibration curve of the standard plasma-porphobilinogen solutions.

The specimens of urine and plasma were examined for their porphobilinogen content within half an hour of collection; before the determination they were stored at 2°C.

RENAL CLEARANCES IN PORPHYRIA

Case no.	Duration		Posture	Clearance of (ml. per min.)			
	From	To		Creatinine	Urea	Endogenous porphobilinogen	Inulin
1	11 A.M.	5 P.M.	R	79	42	117	..
2	7 A.M.	11.15 P.M.	R	..	31	93	..
3	9 A.M.	11 A.M.	S	77	51	70	..
	11 A.M.	1 P.M.	R	92	69	105	..
	1 P.M.	3 P.M.	S	82·5	53	97	..
	3 P.M.	5 P.M.	R	91	73	104	..
4(*a*)	7 A.M.	9 A.M.	R	79	52	84	..
	9 A.M.	11 A.M.	R	64	54	85	..
	11 A.M.	1 P.M.	R	91	67	89	..
	1 P.M.	3 P.M.	R	87	54	89	..
	3 P.M.	5 P.M.	R	80	54	82	..
				Mean 80 ± 9·2	56 ± 5·5	86 ± 2·8	..
(*b*)	Noon	2 P.M.	R	..	..	90	..
	12.30 P.M.	2 P.M.	R	..	..	..	94

In case 4(*b*) the endogenous porphobilinogen clearance and the inulin clearance were measured simultaneously.

R, recumbent. S, standing.

Urea was determined in blood by the method of Van Slyke and Cullen (Hawk et al. 1947). Urinary urea was determined by the hypobromite method (Harrison 1947).

Creatinine.—The methods described by Hawk et al. (1947) were used for plasma and urinary creatinine.

Inulin clearance.—The method used for this was essentially that described by Higashi and Peters (1950).

Results

The results of these experiments are summarised in the table. The mean plasma-porphobilinogen concentrations in cases 1, 2, 3, and 4(*a*) were 1·0 ± 0, 1·0 ± 0·1, 0·6 ± 0·1, and 1·3 ± 0·2 μg. per ml. respectively.

The mean endogenous porphobilinogen clearances of all the patients when recumbent, corrected in each case to 1·73 sq. m. of body-surface area, was 106 ± 28·4 ml. per min.

Discussion

The results suggest that, in these patients, endogenous porphobilinogen was filtered by the glomeruli and not excreted or reabsorbed by the tubules to any significant extent. Creatinine and inulin are known to be entirely excreted by glomerular filtration. In cases 3 and 4(*a*) the simultaneous renal clearances of creatinine and endogenous porphobilinogen were similar. This similarity was less obvious in case 1. The simultaneous renal clearances of inulin and endogenous porphobilinogen in case 4(*b*) were similar. The mean of the corrected porphobilinogen clearance of all the patients when recumbent (106 ± 28·4 ml. per min.) compared favourably with the figure recorded by Smith (1951) for glomerular filtration-rates obtained in mixed groups of males and females (116 ± 28·1 ml. per min.).

The results of the influence of posture on the renal clearance of creatinine, urea, and porphobilinogen in case 3 require comment. Brun et al. (1945) recorded that glomerular filtration, measured by inulin clearance, and renal plasma flow, measured by diodone clearance, became lower when the reclining posture was changed to the passive erect posture, and then rose to their former levels when the patient resumed the reclining posture. These changes took place within 15–30 minutes of the change of posture. White and Rolf (1948) and Epstein et al. (1951) confirmed this influence of posture on inulin clearance, and Ni and Rehberg (1931) and White and Rolf (1948) showed a similar effect on the renal clearance of creatinine. On the other hand, Viar et al.

(1951) did not find any influence of posture on creatinine clearance.

The renal clearance of endogenous porphobilinogen in case 3 was somewhat higher in the reclining position than in the erect position, and the simultaneous urea and creatinine clearances showed a similar effect. These observations do not clarify fully the rôle, if any, of the renal tubules in the excretion of endogenous porphobilinogen, since the clearance of urea, which is slightly reabsorbed by the tubules, and of diodone, which is excreted by the tubules, are also influenced by changes of posture. However, the close correspondence between the changes in porphobilinogen and creatinine output suggest a closely related mechanism of excretion.

Goldberg and Rimington (1954) suggest that, in experimental porphyria in the rat, porphobilinogen is formed in the liver and not at an extrahepatic site. In acute porphyria in man porphobilinogen is found in the same tissues as in experimental porphyria in the rat —i.e., liver, kidney, and plasma. In addition the mechanism of the renal clearance of porphobilinogen in acute porphyria in man appears to be the same as in the rat. This suggests that the reasoning that porphobilinogen is formed in the liver in experimental porphyria in the rat is valid for acute porphyria in man.

Porphobilinogen is a substance of low molecular weight (226) and is pharmacologically inactive ; it can be easily and accurately measured in very small concentrations in urine and plasma. After its injection into rats a maximal recovery of 79% was obtained, of which 75% was found in the urine, while the slight increase of urinary porphyrins reflected a 0·1% conversion of the injected porphobilinogen. Possibly this substance or one closely allied to it might provide a suitable tool for the determination of the glomerular filtration-rate.

The fact that some patients with latent porphyria and acute porphyria in remission may excrete large quantities of porphobilinogen in the urine while they have a determinable plasma-porphobilinogen level should be remembered when an attempt is being made to assess the progress of a case of acute porphyria. It is also clear that clinical interpretations of changes in urinary output of porphobilinogen must take into account changes in renal function as well as changes in the production of porphobilinogen. For example, a decreased output of porphobilinogen can mean either that the patient is getting over his acute attack of porphyria or that his condition is worsening because of renal failure, with,

of course, a lowered glomerular filtration-rate. Plasma-porphobilinogen levels and clearance determinations can be used to distinguish between these two possibilities.

Summary

Renal clearance has been studied in three cases of acute porphyria and in one case of latent porphyria.

The results suggest that endogenous porphobilinogen is excreted in man by glomerular filtration without appreciable tubular reabsorption.

The significance of this finding is discussed.

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